INFORMATION FOR CONTRIBUTORS

GENERAL

All manuscripts should be addressed to the Editor, *Current Science*, P. B. No. 8001, C. V. Raman Avenue, Bangalore 560 080. Submission of an article will be held to imply that it has not been previously published and is not under consideration for publication elsewhere; and further, that if accepted, it will not be published elsewhere. *Three copies of contributions of all categories* are required, with a letter of transmittal giving (i) names and complete addresses of the authors and (ii) title of the contribution and the category in which it is submitted (see below).

Current Science is a multidisciplinary journal and therefore research and review papers of general significance that are written clearly and well organized will be given preference. All papers will be first assessed by a Reviewing Editor. Papers found unsuitable in terms of the overall requirements of the journal will be returned to the authors. The others will be sent for detailed review. *Both solicited and unsolicited material will be reviewed.* Authors of these papers will be notified of acceptance, rejection, or need for revision of the paper. Returned papers cannot be resubmitted.

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General articles (not exceeding 5000 words) discuss current trends in research in a field that will be of interest to readers outside the field; interdisciplinary topics: science policy and administration; or some aspect of the application of science and technology to human needs or the impact of science and technology on society/ecosystems/life. They should include a summary not exceeding 100 words, introductory paragraph(s), brief subheads at appropriate places to point to what follows, illustrations that will help a general reader, and references.

Review articles (not exceeding 5000 words) are expected to survey and discuss recent developments in a field. They should be well focused and organized, and avoid a general, 'textbook' style.

Research articles (not exceeding 4000 words) should report research results of fairly major significance. They should include an abstract not exceeding 100 words, introductory paragraph(s), and brief subheads.

Research communications (not exceeding 2000 words) should contain important findings that are novel and of fairly broad interest. They should include a brief abstract and an introductory paragraph. Text should not be broken up under subheads.

Correspondence includes letters, not exceeding 500 words, that are of general interest to scientists. All letters cannot be published.

Scientific correspondence contains technical comments, including those on articles or communications published in *Current Science* within the previous six months. Letters may be reviewed and edited.

Research news articles are intended to inform nonspecialists about recently published advances or important findings discussed at a meeting. Authors should also send a copy of the paper(s) on which the article is based. Meeting reports should avoid merely listing brief accounts of topics discussed, and must convey to readers the significance of an important advance.

Research accounts articles are intended to be personalized reviews of research from the authors' own laboratory, based on a body of published work. The articles must provide appropriate background to the area in a concise introduction, which should also serve to place the author's work in proper perspective. Articles will normally not exceed 8 to 10 printed pages.

Opinion articles present views on issues related to science and scientific activity. **Commentary** articles should contain expository notes on issues related to science and scientific activity.

Book reviews. Unsolicited reviews will also be considered. Reviews that merely 'list' brief descriptions of the contents cannot be published. Reviews should have 'context' and convey some information about the subject of the book.

Historical commentary and notes inform readers about interesting aspects of personalities or institutions of science or about watershed events in the history/development of science; most are expected to relate to India. Illustrations are welcome. Brief items will also be considered.

MANUSCRIPT PREPARATION

Manuscripts should be typed double-spaced on one side of white bond paper (21 × 28 cm). The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figure legends. The **title** should be brief, specific and amenable to indexing. Not more than five **keywords** should be indicated separately; these should be chosen carefully and must not be phrases of several words. **Summary** and **abstract** should not have more than 100 words and should convey the main point of the paper, outline the results and conclusions, and explain the significance of the results.

Text. All papers should have a brief introduction. The text should be intelligible to readers in different disciplines and technical terms should be defined. Tables and figures should be referred to in numerical order. All **symbols** and **abbreviations** must be defined, and used only when absolutely necessary. Superscripts and subscripts and ambiguous characters should be clearly indicated. **Units of measure** should be metric or, preferably, SI. Methods should, as far as possible, be described briefly in appropriate table and figure legends.

Figures. In the case of line drawings one set of originals (without any lettering) is sufficient, with two copies containing lettering. In the case of photographs good prints are required with each copy of the manuscript; photocopies are not acceptable. Line drawings should be roughly twice the final printed size. The correct orientation should be indicated if not clear.

Photomicrographs and other photographs that require it must have a scale bar, which should be defined clearly in the legend. Primary data should be submitted as far as possible (e.g. actual photographs of electrophoretic gels rather than idealized diagrams).

References should be numbered in the order in which they appear, first through the text and then through table and figure legends. The following are examples of ways of writing references.

1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355–362.
2. Constantine. G., in *Biology of Bats* (ed. Wimsatt. W. A), Academic Press, New York. 1970. vol 1, pp. 319–322.

Acknowledgements should be brief. Footnotes are not allowed except to identify the corresponding author if not the first.

Cover photographs. Good photographs (colour or black and white) that pertain to a submitted paper will be considered for use on the cover. Good *prints* and a legend should be submitted with the manuscript. In the case of a colour picture, a transparency will be required for printing if accepted.

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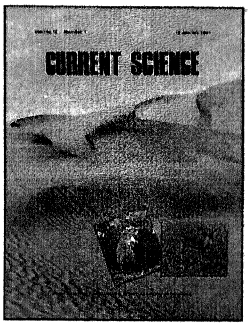

COVER. The barchan dunes, the major cause of soil erosion in the arid zone (Photo: I. Prakash). *Inset left*, The Indian Gazelle, *Gazella bennetti* (Photo: H. C. Bora); *Inset right*, Desert Gerbil, *Meriones hurrianae* (Photo: A. P. Jain). See page 31 for an article on ecology of desert mammals.

Indexed in CURRENT CONTENTS/GEOBASE/CHEMICAL ABSTRACTS

The editors thank Prof. P. Rama Rao, President, Indian Academy of Sciences, Bangalore and Dr A. R. Reddy, Director, Defence Laboratory, Jodhpur for help in bringing out the special section.

In this issue

Thar desert ecosystem

After C. V. Raman held an annual meeting in Rajasthan at Udaipur more than 50 years ago in 1945, the Indian Academy of Sciences revisited the State for the 62nd annual meeting which was held at Jodhpur from November 1 to 3, 1996. Continuing a long-standing tradition, a highlight of the Jodhpur meeting was a symposium of local scientific interest devoted to 'Arid Ecosystem of the Thar desert of Western Rajasthan'. The symposium addressed Thar desert's unique characteristics in the light of modern day science and technology developments.

The Thar desert (drawing its name from a district in Pakistan's Sind province, Tharparkar) is also known as the Great Indian desert and spans an area of approximately 259,000 km^2 in the Indian subcontinent, 69% of which lies in our country's north-western region.

The Thar desert experienced several periodic climatic changes from arid to semi-arid, resulting in the dominance of either the fluvial or the aeolian processes. These processes have dictated much of the present topography. Historical data suggest that, several millennia ago, the total atmospheric precipitation in the Thar was 4 to 5 times higher than at present. The enhanced aridity is believed to be responsible for the decline of the Harappan civilization about four millennia ago. Famines of various kinds, namely Akal (great famine), Jalkal (water scarcity), Tinkal (fodder scarcity) and Trikal (water, fodder and grain scarcity) have been a frequent occurrence. Their dispersion in the Thar is emphasized in a local Rajasthani saying:

'Pug Poongal, Sar Merte, Udraj Bikaner, Bhoolo Chukyo Jodhpur, Thayo Jaisalmer.'

Translated:

'Famine keeps its legs standing in Poongal region in the heart of the desert, its head in Merta, stomach in Bikaner, may move to Jodhpur and stay forever in its home town of Jaisalmer'.

The Thar desert population is mostly rural. The Western Rajasthan population density is 84 persons/km^2 and accounts for 38% of the total State's population. Although the population density is low compared to the other parts of the country, it is relatively high by arid zone standards. The livestock is around 25 million! These figures make this arid zone one of the most densely populated deserts of the world which implies considerable pressure on the region's natural resources. Thus, management of the natural resources assumes great importance. George Joseph of the Space Applications Centre provided in the first lecture of this symposium an analysis of the role of remote sensing in the resource management with special reference to Western Rajasthan.

The climate of the Thar is characterized by high atmospheric temperatures, which often reach 46°C and a strong wind regime with wind speeds up to 30 km/h, leading to a high evaporative demand (1500–2200 mm/year) and frequently to dust-raising winds, dust storms and associated wind erosion. The mean annual rainfall in the Indian region of the Thar desert is 345 mm varying spatially from less than 100 mm in the extreme western part (Jaisalmer district) to over 400 mm along the eastern fringe (Pali, Sikar and Jhunjhunu districts). D. R. Sikka, formerly of India Meteorological Department and Indian Institute of Tropical Meteorology, spoke at the symposium on desert climate and its dynamics.

Hydrologically the Thar desert could be divided into: (i) a region which has its major water source in more humid regions such as the northern districts of Punjab and Haryana and north-western parts of Rajasthan, (ii) plain lands with a primitive (or none) network of streams. The inhabitants of this region are partly dependent on surface water, i.e. harnessing the runoff in the form of village ponds and underground cisterns and largely dependent on groundwater having total dissolved solids of over 3000 ppm at a depth of 40–100 m, and (iii) sloping regions with an integrated stream network (e.g Luni river, its tributaries and many ephemeral channels that remain dry for a large duration). Overall, surface water resources of the Thar desert are extremely unreliable. A considerable fraction of the drinking water demand by both humans and livestock has to be met by drawing groundwater. S. M. Rao of Bhabha Atomic Research Centre who has carried out isotope studies on groundwater in Western Rajasthan presented results of his work at the symposium.

Despite the scarce water resources, about 45% of the total land area of the Indian arid zone is brought under plough every year. Rain-fed agriculture is practical in most areas. Productivity of traditional agriculture on these lands is poor due to low and erratic precipitation, high solar incidence, high wind velocities and poor soil characteristics. These arid areas suffer from shortage of traditional energy sources. Hence it is important to consider development of alternative energy sources such as solar energy, wind power and bio-energetics. S. P. Sukhatme of the Indian Institute of Technology, Bombay, made a detailed presentation of the efforts made and presently underway for generating electricity through photovoltaic and thermal routes from solar energy taking advantage of the high solar incidence over the Thar with the mean annual global solar radiation in excess of 6 kwh/m^2. (Jodhpur, the venue of the Academy meeting is known as 'Sun city' and receives more than 7 kwh/m^2 per day of radiation during April–June.)

Life exists in several forms in the desert. Desert flora are mainly of four types: (i) drought escaping, (ii) drought evading, (iii) drought enduring and (iv) drought resisting. The many varieties of cacti are typical examples of drought resisting desert flora. A wide variety of insects, reptiles, amphibians, birds and mammals enrich the desert fauna. While Mohan Ram of Delhi University delivered, in a separate session, a special lecture on plant life under extreme conditions, Ishwar Prakash, now at the Zoological Survey of India, presented a fascinating account at the symposium on the ecology of the desert mammals.

The five symposium presentations, which appear in a special section of this issue, offer a glimpse of the range of perplexing features and issues pertaining to the Thar desert, in the sustained development of which resides a magnificent challenge to the scientific community.

A. R. Reddy

Essence of heavy hearts

The mammalian heart is a double pump. Each pump is provided with check valves to make it work in one direction and has a squeezable container, the ventricle and a filler, the atrium. William Harvey wrote in 1628: '... the blood in the animal body moves around in circle continuously and ... the action or the function of the heart is to accomplish this by pumping. This is the only reason for the motion and beat of the heart.' The pumping action of the heart results from shortening of muscle in its walls. The contraction of muscle produces the pressure that squeezes blood outward.

An incredible feature of this biological machine is that it is equipped with large factors of safety to meet the strains of every day life. A human heart pumps about 5–6 litres of blood per minute at rest. During strenuous muscular efforts such as running to catch a bus the output can go up to five times in a healthy adult. The heart does this by adjusting the rate of heart beat as well as increasing the volume of blood that comes out during one beat. From beat to beat, the output is regulated in response to muscle stretch. The more a cardiac muscle is stretched, the more vigorously it responds. This mechanism also helps in immediate adaptation when the valves of the heart become leaky due to disease processes. Leaky valves result in more blood in the ventricles, stretched muscles and more forceful contraction. However, continuous hyperfunction or sustained load needs other compensatory mechanisms to cope with the increased stress. Unlike other organs, heart is incapable of adding new units or multiplying its component cells. The basis of its growth is increase in the size of the muscle cells, i.e. hypertrophy. Hypertrophy is a phenomenon of normal growth of the heart. As body mass increases about 20 times from the time of birth to adulthood, there is proportionate increase in the cardiac muscle mass.

Both birth defects and acquired heart diseases can increase the load on the heart by either amplifying the resistance to cardiac output (pressure overload) or increasing the influx of blood into the heart (volume overload). In such situations, heart hypertrophies to increase the strength of contraction. A hypertrophied heart in some pathological conditions may weigh even one kilogram, 3–4 times the normal. Compensatory hypertrophy is a decisive factor for long term adaptation in a variety of heart diseases.

Though initially the hypertrophied heart is capable of meeting the increased work demands, during continuous hyperfunction, the reserves of the myocardial cells are successively mobilized until they are exhausted and function becomes severely impaired. Thus myocardial hypertrophy can be considered as an interface between normal and failing heart.

The hypertrophic response consists of not just an increase in muscle proteins. It is associated with re-expression of foetal genes and several proto-oncogenes. Significant increase also occurs in the number of non-myocyte cells, particularly the connective tissue cells, as well as collagen and other extra cellular matrix components.

Thanks to the use of cultured myocytes, transgenic animals in which hypertrophy is induced and application of recombinant DNA techniques, several questions regarding cardiac growth are being explored. Many questions have been answered and some remain to be elucidated.

What is the signal that transduces wall tension into cardiac growth? How is the signal from the cell surface mediated to the nucleus? What factors initiate and maintain protein synthesis? What is the role played by hormones and growth factors? These questions are addressed by D. S. Reddy in his review article (**page 13**).

There are three reasons for the study of mechanisms of cardiac growth: (i) understanding the circumstances that lead adaptive cardiac growth to pathological hypertrophy and heart failure is expected to lead to a clinical cure for cardiac failure, (ii) knowledge gained on cardiac growth may provide clues to augment cardiac muscle growth in injured myocardium. If cardiac growth can be induced by use of growth factors, it will also be of benefit in modulating long term adaptation of the heart, (iii) the model of compensatory hyperfunction of the heart is viewed as an ideal one for the study of inter-relationship between function and the genetic apparatus, a key element of long term adaptation in physiological systems.

C. C. Kartha

CURRENT SCIENCE

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EDITORIAL

An uncertain future

Support for science, both financial and otherwise, is diminishing in India as government's perceptions about the role of the science and technology enterprise in future development, appear to have changed rather rapidly. Higher education in science is threatened by the prospect of institutions having to raise funds from private sources to meet essential running expenses, even as government slowly abdicates its responsibilities in this area. Research departments and institutions can hardly compete for private resources, with management and marketing institutes and colleges and universities distributing engineering and medical degrees. The heady days of enthusiastic government support for science appear to be over. Unfortunately, the rationale for changing policies is often obscure, although budgetary compulsions are always blamed for across the board, non-discriminatory cuts in all areas of governmental expenditure. The uncertainties of the new year, which coincides with the start of a new Five-Year Plan, put together by a disparate coalition of interests, cast a long shadow on the future of scientific institutions in this country. While the defence science, space and atomic energy establishments will undoubtedly come through unscathed because of their perceived strategic importance, the rest of India's science institutions face difficult times ahead.

In most places, frozen budgets over the last few years have seen an ever increasing proportion of the allocated funds (in some cases as high as 90%) going towards payment of staff salaries and allowances, leaving little or no institutional support for research activities. Individual researchers survive largely on sponsored projects, which are of course easier to obtain and operate in a relatively small number of high profile institutions. Even here the decline is clearly visible. Projects submitted to government agencies lie for increasing lengths of time, unattended. Even more depressingly, release of funds for sanctioned projects proceeds at a glacial pace. The ever present battle for turf, an enchanting pastime for bureaucrats in Delhi, appears to have tilted against the managers of the science departments. Procedural delays are now the rule rather than the exception. Ironically, liberalization is a word that seems absent in the lexicon of bureaucracy, both in Delhi and inside our institutions. The pity is that by slowing the process of procurement of equipment, materials and staff appointments, most projects eventually end up costing more. In recent times, there has been much comment about the gradual aging of the scientific work force. Limited recruitments over several years have resulted in a graying of our laboratories. What appears to have passed unnoticed is that aging is a phenomenon that has also caught up with the middle level management of our science ministries.

Funding and encouragement for science may be at a low ebb, but it is also true that many of our agencies and laboratories are largely devoid of good ideas. Collective enthusiasm, which facilitates problem solving, is conspicuous by its absence. Innovation is rare and hardly encouraged by an unresponsive and conservative environment. The scientific establishment today suffers from poor public relations; an inability to enhance the public (and political) perception of what science in India has achieved and even more importantly the crucial contributions that it will make in the future. The herbal petrol fiasco highlighted dramatically the public craving for quick, magical fixes to mounting problems. The faith in conventional science obviously, does not run too deep.

Most non-performing wings of government get a far larger share of the public cake than the science departments. Scientific institutions most often struggle with limited budgets and unrealistic expectations of what can be achieved. Science, technology and higher education need a broad based lobby, which will prevent skewed priorities and ill thought out development strategies from cutting at the roots of our scientific and technological development. It is also clear that scientific institutions must devise mechanisms to shed some fat. Leaner and fitter organizations are more likely to cope successfully with an uncertain future.

P. BALARAM

Herbal medicines – some more reports

Several problems associated with the use of Herbal Medicines (HM) were discussed earlier in this column. Presence of toxic materials in HM, quantitative variation of the constituents from batch to batch of the plant products and alleged admixing of allopathic medicines with the herbal formulations by the manufacturers have raised serious concern among the medical scientists about safety of the patients[1,2]. A couple of subsequently published reports, highlighted here, reveal some other difficulties in the safe use of HM.

A herbal stimulant named Ma Huang claimed to be a mood enhancer and an aphrodisiac has recently been attributed to the death of 15 persons in the US[3]. In another case a person, who had no history of mental illness, became paranoid after taking Ma Huang for 10 days and jumped from an upstairs window with the fear that he would be killed by some supposed attackers. Fortunately he sustained only minor injuries. The formulation is known to contain ephedrine which is used as a nasal decongestant and bronchodilator but can cause psychosis among other complications when used in overdose. There was no mention of ephedrine in the leaflet of the product. On being contacted, the manufacturers failed to answer how much ephedrine was present per tablet[4]. This lack of information in the product literature of HM is a major problem for the patients throughout the world. Allured by the advertisements in the public media (as it happened in the second case) they buy and take HM and sometimes incur a lot of additional problems.

There is also reason to be worried about the microbiological and parasitological quality of the HM. A couple of months ago, some researchers detected harmful parasites in a sample of fish used to give an Ayurvedic medicine to thousands of asthmatics every year on an auspicious day of June[5].

It must be emphasized that nowhere in the world do the scientists deny the immense potential of the HM to complement the western medicines, but the question of safety of the patients cannot be relegated to the background. In this context it is worthwhile to note the comments of a reviewer 'A hundred years ago a pharmacist might sell you opium mixture, very likely adulterated. Now all over-the-counter medicines are regulated, and it is time "alternative" ones were as well'[6].

1. Chattopadhyay, M. K., *Curr. Sci.*, 1996, **71**, 5.
2. Bhattacharyya, P. C., *Curr. Sci.*, 1996, **71**, 341.
3. Josefson, D., *Br. Med. J.*, 1996, **312**, 1441.
4. Doyle, H. and Kargin, M., *Br. Med. J.*, 1996, **313**, 756.
5. Madhavi, R., Jhansilakshmibai, K. and Murugesh, M., *Curr. Sci.*, 1996, **70**, 350–351.
6. Savage, A., *Br. Med. J.*, 1993, **307**, 332.

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Biobatteries

The report on 'Biobatteries' was not properly vetted by *Current Science* before being published (*Curr. Sci.*, 1996, **71**, 92–93). To say the least, there is little or no novelty in that report, the 'biobattery' developed with fruit juices being just a version of the simple Daniel cell. The extensive references to biomass conversions included in the report notwithstanding, one can easily see, from an elementary knowledge of how electrochemical cells work, from what can be gathered from 'How Things Work' encyclopaedias or even from secondary school science classes, that the electric energy is drawn, not from the 'juices' through some direct biochemical process, but from zinc, when it is corroded by those juices, the energy having been put into the zinc, in the first place, when it was smelted from its ore by reduction to the metal stage through a high-energy metallurgical process*.

Years ago, when the transistor radio was new, it was demonstrated that one such radio, equipped with earphones, can be operated by means of a 'cell' made of copper and zinc American one-cent coins separated by a piece of newspaper moistened with saliva (1943 one-cent coins were minted out of zinc, instead out of the usual copper, in order to conserve the latter for the 'war effort'; the few copper 'pennies' that were minted in 1943 are now numismatic collector's items, worth millions of dollars, I am told!). The principle was, of course, that of the Daniel cell – as zinc ionizes into the 'juice' or saliva (abundantly available in Indian streets!), the released electrons flow through the wire or connected apparatus from the cathode (zinc) to the anode (copper). In modern 'lithium batteries' lithium metal serves, instead of zinc. The function of the 'juice' is to act as an ion transport medium with an 'overvoltage' sufficient to prevent the electrons from engaging in a direct reaction near the zinc, without producing a 'current' said to 'flow' from the anode to the cathode. The reaction ensuing the arrival of the electrons at the anode is one of producing hydrogen by a process of reduction. The current tends to fall off, however, when the copper gets covered over by a film of gaseous hydrogen. This is the so-called 'polarization' of the simple Daniel cell. No wonder, then, the authors find it necessary to clean the electrodes (especially the copper anode) 'frequently' in order to restore the potential to the 'fresh' value (incidentally, what is the meaning of values of current (in mA) given in the tables? Were they measured with the electrodes 'shorted'?).

'Polarization' is avoided by oxidizing off the hydrogen. In a Leclanche cell, where the 'electrolyte' is moist ammonium chloride, the oxidizing agent is manganese dioxide packed around the anode, a graphitized carbon rod as in a

---

*These comments apply also to a 'battery' using slurry from a biogas generator, described by the CIAE, Bhopal; see Mital, K. M., *Biogas Systems – Principles and Applications*, New Age, New Delhi, 1996, pp. 285–286.

torch cell you throw away when once the zinc casing has mostly dissolved away.

I am not certain of the ergonomics of the 'biobattery' but it would really have been impressive if the authors had considered the possibility of methane being cogenerated with the hydrogen near the anode and managed to demonstrate either that it happens or that it does not. If methane is formed at all under the relatively aerobic conditions of the 'biobattery', it would have been an 'advance' on (not really a mimic of) the as yet poorly understood process of biomethanation which takes place only under the exclusively anaerobic conditions of the rumen (of cows, sane or insane) or biogas digestor. Or, there would have been real novelty to the authors' efforts had their aim been to isolate and immobilize an oxidizing enzyme from a natural source, one that utilizes oxygen from the air to oxidize the hydrogen produced near the anode. Commercially viable or not, replacing manganese dioxide by such an enzyme for overcoming 'polarization' would have been a real breakthrough for developing a 'biobattery'. Maybe, the electric eel has all the answers – it utilizes energy from combining what it ate of the prey that it has stunned with an electric shock with the oxygen dissolved in seawater, having met the challenge of inventing a true 'biobattery' millions of years ago!

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This is with reference to the article 'Biobatteries to utilize bioenergy from fruit and vegetable wastes'. The item is misleading because the source of electrical energy in these batteries is the dissolution of Zn. The standard potential for the CU/Zn couple is 1.1 V and it is not surprising that when these two electrodes are immersed in a paste of vegetable or fruit waste, a current is observed. The readers of *Current Science* deserve better.

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NEWS

R&D efforts in industry – National awards for 1996 presented

In order to provide recognition to the efforts of industry towards innovative research and technological development, the National Awards for R&D efforts in industry were instituted in 1987 by the Department of Scientific & Industrial Research (DSIR). These awards are in the form of silver shields and are presented along with citations at the inaugural session of the 'National Conference on in-house R&D in Industry', held annually by DSIR.

Professor Yogendra Alagh, Union Minister of State for Science & Technology presented the shields on 10 December 1996 in New Delhi to the following seven winners of awards for 1996:

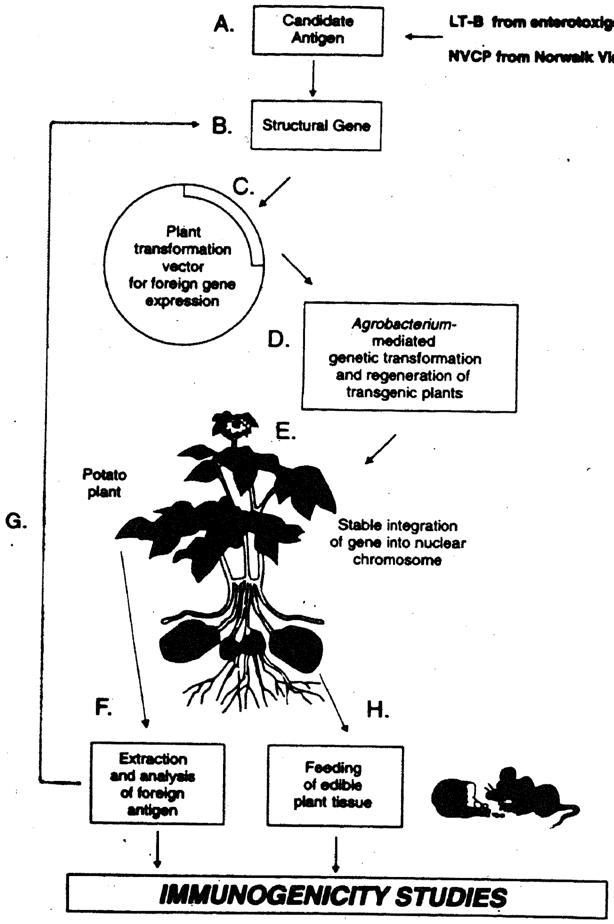
- **Armour Polymers Limited, Mumbai**, for the development and commercialization of the amoxidation process for the manufacture of cyano pyridines.
- **Hetero Drugs Ltd., Hyderabad**, for overall bulk drugs and intermediates.
- **Lamco Lightning Arrester Mfg. Company Pvt. Ltd., Hyderabad**, for the development and manufacture of lightning arrester disconnectors.
- **Patwa Kinarivala Electronics Ltd., Vadodara**, for the development of a range of instruments and electronic control systems for the textile industry.
- **Titan Industries Limited, Hosur**, for the design, development and commercialization of ultra-thin quartz analog watch movements.
- **Ankur Seeds Pvt. Ltd., Nagpur**, for breeding superior hybrids of cotton, okra, chilli, brinjal, cucurbits and sunflower.
- **Jain Irrigation Systems Ltd., Jalgaon**, for fully absorbing technology, imported for micro-irrigation and sprinkler systems, and improving it by their own R&D.

RESEARCH NEWS

Vaccine production in transgenic plants

P. Suprasanna, T. R. Ganapathi and P. S. Rao

The development of novel techniques of tissue culture and molecular biology to genetically transform plant species has revolutionized plant biology, and there is an enormous interest to design and produce plant species with desirable characteristics such as insect resistance, disease resistance, herbicide tolerance and delayed fruit ripening. The notable achievements in these areas led to explore the possibilities of employing transgenic plants with modified biosynthetic potential for new products[1]. Biologically active peptides and proteins have many potential pharmaceutical applications including use as vaccines, immuno-modulators, growth factors, hormones, blood proteins and enzymes[2]. There has been a recent interest in this direction in producing functionally active proteins, peptides of medical importance, in transgenic plants. On a global scenario, the developing countries are often the target of several infectious diseases. Preventive medicine (for example, vaccines) has proceeded rapidly in the last decade as biotechnology has been applied in several areas. These medical


A.
Candidate Antigen
LT-B from enterotoxigenic *E. coli*
NVCP from Norwalk Virus
B.
Structural Gene
C.
Plant transformation vector for foreign gene expression
D.
Agrobacterium-mediated genetic transformation and regeneration of transgenic plants
E.
Potato plant
Stable integration of gene into nuclear chromosome
G.
F.
H.
Extraction and analysis of foreign antigen
Feeding of edible plant tissue
IMMUNOGENICITY STUDIES

A. Optional factors for choosing a candidate antigen
- Purified protein is sufficient to promote immunity to the disease.
- Purified protein is orally immunogenic.
- The gene for the protein is cloned.
- The protein maintains its antigenic epitopes and assembles into its native form when expressed in a heterologous system.

B. Factors to consider in selecting a gene for plant expression systems
- Modification of the gene with plant specific regulatory elements.
- Analysis and reconstruction of antigen-coding sequence for optimal plant expression.

C. Create a plant transformation vector

D. Transform plant tissue

E. Generate plants for analysis

F. Analyse recombinant protein for antigenic epitopes and proper assembly within plant tissue

G. Optimize production of recombinant protein in plant tissue by redesign of gene or transformation vector

H. Test oral immunogenicity of the vaccine-containing transgenic food
- Test for induction of an immune response to the recombinant protein.
- Challenge immunized animals (if possible) with the disease to determine if the candidate vaccine can induce a protective immune response.

Figure 1. Production of candidate vaccines in plants is shown schematically along with factors to consider for various steps. (From Richter *et al.*[5]; Reproduced with permission from Dr C. J. Arntzen.)

advances are not likely to have significant impact in developing countries because of prohibitive costs of production and delivery of the recombinant proteins which comprise the new vaccines (Arntzen, pers. comm.). The economic conditions and inaccessibility to world markets also limit the use of vaccines for immunization to large population. In this regard, the concept of vaccine production in transgenic plants assumes greater significance for a relatively low-cost, agriculture-based system, rather than the sophisticated and expensive cell culture and fermentation-based vaccine production.

The concept was first introduced by Charles J. Arntzen and his group in a report of vaccine production in tobacco plants genetically engineered with plant-derived recombinant hepatitis B surface antigen (rHBsAg) (ref. 3). In this study, they have demonstrated that the HBsAg was expressed in tobacco and the partially purified protein when injected into mice, elicited antibody response, similar to that obtained with 'recombivax' (a commercial hepatitis B vaccine). This study clearly demonstrated close-relatedness of the antigens and also the potential of producing immunologically active vaccines in transgenic plants.

Potential recombinant vaccines for viral and non-viral organisms have been produced in transgenic plants. Norwalk virus causes epidemic, acute gastroenteritis. The recombinant capsid protein (NVCP) was expressed in transgenic tobacco leaves and potato tubers[4]. The capsid protein could be extracted from tobacco leaves in the form of 38 mm Norwalk virus-like particles. rNV expression levels up to 0.23% of total soluble proteins in tobacco leaves and up to 0.37% in potato tubers were obtained. The partially purified recombinant NVs from tobacco were given orally, and potato tubers expressing NVCP were fed to mice. In both the cases, stimulation of antibody (IgG) specific to rNV was noticed. This study also demonstrated that oral consumption of recombinant plant tissues can evoke specific immunoglobulin production, furthering the concept of 'edible vaccines'.

In another study[5], tobacco and potato were transformed with genes encoding the binding subunit of *E. coli* heat labile entero-toxin (LT-B). Worldwide, enterotoxigenic *E. coli* and *Vibrio cholerae* cause acute watery diarrhoea by colonizing the small intestines. Transgenic plants having the LT-B gene expressed the protein similar to the bacterial protein. Transgenic tubers when directly fed to mice, elicited the development of both serum IgG and mucosal IgA antibodies to LT-B, in contrast to no LT-B specific antibodies in mice fed with non-transformed tubers.

Research on transgenic plant-based production of vaccines has just begun and already studies on optimization of accumulation of recombinant proteins in edible plant tissues and feasibility including economics of such approaches are being made. These investigations generate a hope towards developing low-cost edible vaccines from plants that are commonly available, compared to expensive recombinant vaccines. In order to be applicable to large section of population in a edible and un-cooked form (to ensure, there is no denaturation), production of vaccines in candidate crops like banana can be invaluable to many tropical countries in the developing world where these are grown, richly available at lower costs and consumed as staple food.

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SCIENTIFIC CORRESPONDENCE

Assessment of plant bio-diversity at Kalakad–Mundanthurai

T. Ganesh *et al.* (*Curr. Sci.*, 1996, **71**, 379–392) have generated interesting phytosociological information for the Tiger Reserve in the Agasthyamalai range.

While highlighting the importance of quantitative analysis in an area of 3.82 ha, the pioneering work of Champion[1] and Puri *et al.*[2] is referred to as based on qualitative criteria such as physiognomy and dominant species, ignoring quantitative data.

Champion's treatise on the vegetation types came out in 1936, later on revised by Champion and Seth in 1968 (misquoted as 1936 in the reference). This study covered the entire sub-continent. The idea was to come out with a classification good for the country as a whole, with spot descriptions. Significantly, Champion chose to call his *magnum opus* 'a preliminary survey'. Had he confined himself to small plots with quantitative statistics, the concept of which barely existed in his time, perhaps, the publication would not have yet seen the light of the day.

Contribution of Puri *et al.* of a later date (1983) relies on the vegetation maps of the French Institute at one millionth scale based on the criteria of dominance–abundance–fidelity of species. The series recognized for the Kalakad–Mundanthurai region is *Cullenia–Mesua–Palaquium*. It is within this series that the quantitative study has established *Cullenia–Alagaia–Palaquium* sub-type considering an area of 3.82 ha.

The question is for how large an area would this sub-type remain valid, latitudinally and altitudinally? How many subtypes founded quantitatively go into one series, viz. *Cullenia–Mesua–Palaquium* of the southern part of the Western Ghats? How many hundreds (or thousands) can be counted in Peninsular India? What is good for a 4 ha study carried out in considerable detail may not be manageable at the level of the sub-continent. The question of scale should not be lost sight of.

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Reply by T. Ganesh et al.:

We recognize the pioneer and monumental contributions of Champion[1] and Puri *et al.*[2] to the description of forest vegetation in India, but reiterate that plant ecologists in India must move beyond qualitative description of vegetation, particularly in light of the current need for assessment and monitoring of biodiversity. Our paper represents an effort towards quantification of tree diversity in an area that is poorly known, but has a high level of biodiversity. Incidentally, Meher-Homji and his colleagues, notably Pascal[3] from the French Institute, themselves have been pioneers in collecting quantitative data on the distribution of tree species in plots throughout the Western Ghats.

We also wish to point out that the 3.82 ha that Meher-Homji refers to is a sub-sample of an area over 100 ha. Nevertheless, his question for how large of an area would this sub-type remain valid is an appropriate one. Although we recognized the forest type as *Cullenia–Aglaia–Palaquium* type, we did so in the context of types and sub-types established by Champion and others. We now feel that the concept of types and sub-types based on dominance of certain species is of limited utility, and may not be valid in many cases. Plant communities change over time and space. The static description of vegetation does not capture the dynamics of natural communities. Understanding dynamics is key to long-term conservation of biodiversity. In fact, one of the points of our paper is that our results do not conform to the vegetation described for the area because the vegetation is expected to vary over small spatial scales. Therefore, quantitative studies are required to document patterns of diversity and long-term monitoring is essential to understand dynamics. Regrettably, in trying to make our point about changes in community structure over space and time, we fell into the trap in claiming the existence of another subtype. Thus, we are very grateful to

Meher-Homji for providing an opportunity to not only clarify the points raised, but also to state our position concerning vegetation types.

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Electrostriction effect and solvent engineering

Anil Kumar on application of ion–solvent interaction and thermostability of DNA duplex in ionic solution (*Curr Sci.*, 1996, **71**, 289) highlights many important findings which bear relevance not only to DNA but are very much true for proteins and enzymes. The kinetic data presented clearly explain the thermostability acquired by DNA in ionic solution. Interestingly, similar role of electrostriction effect is indicated from several reports on enzyme stability by soluble additives, e.g. salts, sugars and sugar alcohols.

Nowadays the area of thermostability of enzymes is a challenging field for chemists, biochemists, biotechnologists and industrial microbiologists. Lot of work has been directed to achieve this by stabilization of native confirmation of protein in aqueous environment. T_m of lysozyme and ribonucleases and many other enzymes has been raised using sugars and polyols. Thermolabile enzyme preparations have been stabilized by high ionic strength solutions. The role of viscosity, intensification of the intramolecular hydrophobic interaction within the protein reduced volume and increased surface tension has been advocated in protein solvent and additive interactions[1].

The possible role of electrostriction (ES) effect leading to a hydrophobic environment in an aqueous system in enhancing the shelf life of enzyme, altering substrate specificity and rate of reaction, opens up new vistas in solvent engineering of enzyme mediated reactions.

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Anil Kumar replies:

Gupta and Saxena have offered valuable information on the role of electrostriction in explaining several biological processes. We often stress, in the seminars and symposia about effective and fruitful collaboration among biochemists, microbiologists and biophysicists. The time has now come that we, in India define the areas of collaboration and intensify collective efforts to work on the solvent engineering of enzyme-mediated reactions.

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Dengue haemorrhagic fever epidemic in Delhi

Dengue was endemic in India, but dengue haemorrhagic fever (DHF) is of recent origin, first reported[1] from Calcutta in 1963. Since then DHF and dengue shock syndrome (DSS) have been occurring in India sporadically and entering urbanized and industrialized settlements. DHF/DSS epidemics have also been reported from purely rural villages in Maharashtra, Karnataka, Tamil Nadu, Delhi, etc. Urbanization and rural water supply are the principal reasons for *Aedes* dispersal. So far there have been 70 reported outbreaks in the country and the 1996 epidemic was preceded by 6 outbreaks in Delhi. All four serotypes (Den-1, Den-2, Den-3, Den-4) occur in India. In the 1996 epidemic Den-2 virus has been isolated by Pradeep Seth at the All India Institute of Medical Sciences, New Delhi and Kalyan Banerjee at the National Institute of Virology, Pune. The epicenter of DHF was south Delhi affecting all communities, and later cases were reported from almost all localities. In this epidemic beginning in August, the government has reported 8900 cases and 375 deaths due to DHF. Reported cases may be the proverbial tip of the iceberg. Dengue fever and deaths are also being reported from neighbouring towns in Haryana and Uttar Pradesh. Dengue now occurs across the country with increasing reports of DHF/DSS. Army's help was sought, but ironically deaths and large number of admissions due to DHF were also reported from the New Delhi military hospital. The epidemic has receded with the onset of winter but DHF is known to become endemic, and might resurface periodically. In Delhi *Aedes aegypti* breeds profusely with larval index from 20 to 40 or more in many localities, particularly during the transmission months following rains. Breeding opportunities for *Aedes aegypti* are enormous and evenly spread in most Delhi in the form of desert coolers, water storage tanks, leaking water supply, fountains, wells, tire dumps, and rain water collection on roof tops and a variety of receptacles. Vector control under the urban malaria scheme is erratic and limited to some localities by way of spraying of larvicides in surface drains, temephos treatment of water tanks and thermal malathion fogging. Field operations are wanting and mainly targeted to control *Culex* breeding. Malaria is endemic in Delhi and the vector *Anopheles stephensi* and *Aedes aegypti* largely share similar breeding habitats. DHF epidemic coincides with *P. falciparum* prevalence making diagnostics a problem. Dip-stick test[2] for *P. falciparum* would be desirable. While malaria is treatable, there is no specific treatment for viral infections. In dealing with DHF preventive vector control is the key to success. This is attainable by a well-organized *Aedes* control programme. The control of *Aedes aegypti* by chemical methods like spraying and fogging or ULV application is not productive and sustainable[3], except epidemic control[4]. *Aedes* control requires strong entomological support which is weak in the states and almost non-existent in the municipal corporations and smaller bodies[5]. The disease has come to stay and governments would do well by emphasizing community participation and taking help of legislative measures. The approach to DHF control should be community based directed towards species sanitation and learning from the DHF endemic countries.

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Sex and the single X

In mammals, including human, it is believed that the Y chromosome by its presence or absence exerts a series of actions upon which develop the sexually dimorphic characters. Although a number of genes in this cascade of binary decision might be autosomal or X-chromosomal, there must be one or more gene(s) on the Y chromosome to govern the process of testis determination if the male development is a dominant pathway.

SRY is the only gene that is required on the Y chromosome for testis determination as the introduction of transgenic *SRY* alone into chromosomally female mice results in male development[1]. Recently a few autosomal genes have been identified that are probably involved in testis development. For example, mutation in *SOX9* gene is associated with XY sex reversal in human[2] and the null mutant for the gene *SF-1* shows severe abnormalities in the gonadal development of mice[3]. In contrast to our current understanding of the process of male development in mammals, the genetic cascade of the female pathway is almost completely unknown. It is possible that in some of the XY females, a 'loss-of-function' mutation in a gene that inhibits ovarian development (in males) could have been the cause for sex reversal. Although chromosomal deletion in 9p and 10q is associated with such XY females, interruption of testis development was viewed as the cause for sex reversal having led to the female development by default[4]. However, that the ovarian differentiation may not be a passive one is indicated by the fact that in wood lemmings, rearrangement in an X chromosome induces the development of reproductively active XY females even in the presence of a normal Y chromosome[5]. More interestingly, in two species of mole-vole, sex determination is in the absence of Y chromosome (XO male; XX female)[5,6]. The first indication for involvement of an X chromosomal gene in human sex determination was provided by the identification of a family with an X-linked mode of inheritance of 46,XY sex reversal[7]. Demonstration of duplication in short arm of the X chromosome (Xp21 region, called the *DSS* locus) in such 46,XY females raised the interesting possibility that the duplicated X chromosomes cause XY sex reversal by expressing double dose of a gene normally subject to X inactivation[8]. However *DSS*-negative 46,XY males develop testis and show normal external genitalia, indicating *DSS* is not involved in testicular development[8]. Therefore, it is puzzling to imagine how the inactivation of X chromosome and the dosage activity of this locus control gonadal development.

Recently Jimenez *et al.*[9] discussed an appealing model for the dosage effect of *DSS* speculating a female determining function for the *DSS*. It is proposed that in normal female the *DSS* down regulates autosomal genes involved in male development (e.g. *MIS*) but the autosomal factors are not inhibited in the 46,XY males owing to the suppressive action of *SRY* on *DSS*. Thus, testis development implies inhibition of female pathway genes and for the ovarian development, the male pathway genes must be suppressed. Therefore, deletion of *DSS* does not affect male development. However the duplication of *DSS* in XY individuals [46,XY *dup*(Xp)] renders the level of SRY protein insufficient to suppress the two active doses of *DSS*, thus leading to development of female phenotype. It is likely that such a dosage effect might have been the primitive mechanism of sex determination in mammals and that *SRY*'s role is a recent addition because some of the events of sexual differentiation in the primitive mammals, marsupials, are based on the ratio between X chromosome and autosomes[10]. In fact the effect of dosage in sex determination has earlier been shown for birds (the group that diverged from the same ancestral stock of mammals) although here it is based on the Z chromosome to autosome ratio[11]. Therefore, Chandra, by extending his original hypothesis on dosage effect in mammalian sex determination, viewed the absence of dosage compensation for Z chromosome as a key factor in the avian sex determination[12].

While the *DSS* dosage model[9] is attractive because it resolves the paradox of 46,XY *dup*(Xp) sex reversal and offers an evolutionary perspective as stated above, but the support for this model at present is very little. Moreover there are a few points to be considered before suggesting such a sweeping role for *DSS*. First, there is no evidence for *SRY* to be an inhibitor of *DSS*. Should that be so in all the mammals then, in the mole-vole *Ellobius*, which lacks Y chromosome and *SRY* (ref. 6), there should be no males. Likewise, in human while *DSS*-negative males are normal, the effect of such a genotype on genetic female is not clear due to the random X-inactivation. Moreover the proposed negative regulatory role of *MIS* (a male pathway gene) on 'female pathway genes' and of ovarian germ cells on 'male pathway genes' in sex differentiation is questionable since in the *MIS*-deficient mice, testis is spermatogenically active[13], and the gonadectomized females are phenotypically normal with little obvious effect of the absence of germ cells[14]. A role for *DAX-1* (the gene which falls in the *DSS* region and code for a nuclear receptor) in gonadal determination is not clearly established since no rearrangements in *DAX-1* could be detected in thirty 46,XY females[7]. Nevertheless, the occurrence of *DSS*-deleted males and duplication of *DSS* in 46,XY females emphasize that *DSS* is necessary for ovarian development and may be repressed in XY individuals.

The recent progress in the identification and characterization of genes like *SF-1*, *SOX9* and *WT1* (ref. 16), which show multiple functions in addition to their role in gonadal differentiation, reveal a multi-factorial hierarchy in the cascade of gonadal determination. Moreover, testis development in XX–XY chimeras with atleast 25% XY cells[17], high incidence of XX sex reversal for *SRY*-transgenes in homozygous condition rather than in hemizygous, correlation of incomplete penetrance of *SRY*-transgene with a lower level of its transcripts[16], and duplication of *DSS* and XY sex reversal[8], all suggest a concentration threshold for sex-determining factors in the gonadal development. Therefore, it is reasonable to explain that, instead of assuming the 'one-gene one-function' role of *SRY* in suppressing only the *DSS*, there may be opposite and competitive (indirect?) effects of *SRY* and *DSS* likely on one or more critical factor(s) whose concentration may determine the gonadal switch. Doubtless the switch must function in association with other factors as well as their developmental timing. Further experiments including the construction of animals carrying additional copies of *DAX-1* will be required to understand the process of ovarian determination and the role of *DSS* in it. It may also lead to the discovering of putative additional factors. Moreover the 'default' pathway of ovarian development in mammals should be reconsidered to identify critical genes for ovarian determination, if any.

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Cellular and molecular biology of cardiac hypertrophy

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Cardiovascular system at the cellular level adapts to chronic haemodynamic changes by altering gene expression. Cardiac cell-specific expressions of many contractile protein genes are regulated at the transcriptional level by the interaction of activator proteins with specific *cis*-acting sequences. A number of positive and negative *cis*-acting elements from upstream of the basal promoter region and some of their *trans*-acting protein binding factors have been characterized. Recent epidemiological studies implicated left ventricular hypertrophy (LVH) as a premonitory symptom of mortality from many cardiovascular complications. Although cardiac hypertrophy by a chronic haemodynamic overload *per se* is not a pathological rather biological adaptive response of heart, heart failure as a final form of hypertrophy is an obvious disorder producing high mortality. Qualitative changes in the actin-myosin genomic expression such as re-expression of fetal isoforms, through the induction of immediate-early genes (proto-oncogenes), allow the hypertrophied cardiac fibre to develop a normal active tension to the sustained load at the cost of its maximal shortening velocity, thus inducing a pathological state. Multiple lines of evidence support the existence and functional integration of a variety of humoral factors within the heart, and may participate in the genesis of LVH. The renin–angiotensin system (RAS) has been suggested to play a pivotal role in the regulation of cardiac cell growth and LVH. Other humoral mechanisms such as sympathetic nervous system and endothelin vasoconstrictors have also been implicated. Thus, the elucidation of the cellular and molecular biology of normal cardiac growth, development and hypertrophy may hopefully lead to the development of novel therapeutic strategies to cardiac failure.

CARDIAC hypertrophy (CH) is the pathophysiological adaptation of the heart to chronic mechanical overload, in conditions such as essential hypertension, valvular heart diseases and myocardial infarction. Recent epidemiological studies implicated left ventricular hypertrophy (LVH), detected as an increased ventricular muscle mass, as a premonitory symptom of mortality from many cardiovascular complications. Although hypertrophy by a chronic overload *per se* is not a pathological rather biological adaptive response of heart, heart failure as a final form of hypertrophy is an obvious disease causing high mortality. Qualitative changes in genomic expression allow the hypertrophied cardiac fibre to develop a normal active tension to the increased load at the expense of its maximal shortening velocity (V-max). These changes are preceded by the temporary expression of proto-oncogenes, isogenes of proteins of contractile apparatus and the re-expression of 'fetal' isoforms.

In recent years it has become clear that a variety of hormones are present in the heart and may participate in genesis of LVH. Mechanical load or stretch in cardiac tissues has been identified as a growth stimulus. For several years, humoral mechanisms such as the renin–angiotensin system (RAS) and the sympathetic nervous system have been speculated to play a role in the regulation of cardiac cell growth and hypertrophy. Extensive studies from our laboratory[1–8] have demonstrated the contribution of angiotensin systems in the pathogenesis of several cardiovascular disorders, particularly the ventricular hypertrophy and failure. Other factors that have been postulated include endothelins, thyroxine, cortisol and various peptide growth factors[2,3]. Atrial natriuretic factor (ANF), nitric oxide (NO), and specific prostranoids may negate and balance the actions of various ionotropic agents[3], and contribute to the cardiovascular homeostasis. The elucidation of the cellular, molecular biology of normal cardiac growth, development and hypertrophy is of vital interest to cardiovascular biologists to reveal the pathobiology of congenital cardiovascular defects, maintenance of normal structure and function, and pathogenesis of cardiac muscle failure, which permits to develop newer therapeutic strategies to cardiac failure. As a consequence of the newly developed techniques of recombinant DNA and genetic manipulation, as well as molecular biology based antisense oligonucleotide probes[9], the understanding of the cellular and molecular biology of the heart, particularly hypertrophic growth is receiving widespread attention in the field of cardiovascular medicine. The present review briefly updates our understanding of cellular and molecular biology of cardiac hypertrophy and proposes potential pharmacological interventions for the prevention and regression of left ventricular hypertrophy.

Cardiac growth and development

During the last decade, a variety of morphological, physiological and biochemical studies have provided an improved definition of the alterations in myocardial structure and function that herald the sequential stages of cardiac growth and development. Cardiac growth process can be divided into three categories: embryonic cardiogenesis, post-natal development and hypertrophic adaptation[10]. The first two occur in normal developmental process, and hypertrophic growth is induced by hemodynamic overload.

Cardiogenesis starts with a proliferation of mesodermally derived progenitor cells and commitment to the cardiac muscle cell lineage[11,12], which is followed by differentiation to form a primordial heart tube. The heart tube consists of an outer layer of cardiac muscle cells and an inner layer of endocardial cells. Subsequently, the heart tube acquires an S-looped configuration. At this stage, bulbar, ventricular, atrial and sinus venosus can be distinguished[13]. Eventually, organogenesis proceeds through a series of involutions of heart tube and the onset of septation resulting in distinct cardiac chambers, outflow tracts and the acquisition of regional-specific properties of atrial, ventricular and conduction system cells. The subsequent enlargement of embryonic heart is largely due to myocyte multiplication, which continues until shortly after birth, when cardiac myocytes lose their proliferative capacity and acquire the terminally differentiated phenotype of adult cardiac muscle cells[14]. Acquisition of the divergent morphological, electrophysiological, biochemical and contractile properties of specialized cardiac cell type is largely because of the activation of regional specific programme of gene expression[15].

Recent studies, applying immunohistochemical and *in situ* hybridization techniques to genetic markers have provided some clues to the sequence of molecular and cellular events in cardiogenesis[16–19]. Genetic markers may be viewed as the final step in the molecular pathways that control the complex processes of commitment and regional specifications. Cardiogenesis related to the time of occurrence of atrial and ventricular specificity has attracted much attention. Lyons *et al.*[20] have provided initial insight into the events that lead to cardiac muscle gene expressions into atrial and ventricular specification in adult myocardium. Genes that are differentially expressed in atrial and ventricular tissues like ANF, myosin light chain-1_A (MLC-1_A) which are atrial specific and myosin light chain-1v (MLC-1v), β-myosin heavy chain (β-MHC) which are ventricular specific, are candidates to be used as markers to study cardiogenesis[13]. During the earliest stages of cardiac development ANF and MLC-1A genes are co-expressed in both atria and ventricles but at later stages they are restricted to atria only[18,20]. These atrial genes accomplish their regional specificity through their negative regulation in the non-expressing ventricular region[21]. Ventricular MLC-1 gene is co-expressed in all cardiac chambers in the early embryonic heart, and then is selectively down-regulated in the atrium during the later developmental stages. Analysis of day 8 murine embryo shows that MLC-2 gene is expressed exclusively in the ventricular region of the heart tube, suggesting that ventricular specification of heart occurs at the earliest stages of cardiogenesis[22-24].

In addition to the development of atrial and ventricular chambers, cardiogenesis also leads to the development of valvular structures derived from endocardial cushions, and differentiation of highly specialized pacemaker from the conduction system. Limited information is currently available regarding the pathways that regulate gene expression in these specific cardiac cells. In endocardial cushion, β-transforming growth factor[25] and a specific isoform of the fibroblast growth factor receptor are co-expressed[26]. Recent studies have documented the expression of 'Id' a negative helix–loop–helix regulator, in the growing endocardial cushions[27]. The expression of these genes in the subsequent development of valvular structures and septation events is still obscure. Neural genes, such as neurofilament-I perhaps expresses them into SA and AV nodal cells[28], and it suggests that the conduction system may either be derived or influenced by the neural crest during cardiogenesis[29]. Identification of the positional and molecular clues of gene expression in developing heart will enable us to understand the mechanism of malformations of cardiac structures.

Around birth important switches in gene expression such as postnatal changes in contractile proteins start operating[30,31]. During the fetal development β-MHC is the most abundant isoform, but α-MHC abruptly increases following birth and remains as a predominant isoform during the adult stage. The induction of α-MHC coincides with increase in circulating thyroid hormone after birth[31,32]. Alterations in the thyroid status have been shown to be important for the postnatal protein isoform switching[33]. Promoter region of α-MHC gene contains a binding site for thyroid hormone[34,35]. Binding of thyroid hormone to this nuclear receptor induces a conformational change and ensues the binding of this complex to its DNA binding-site. This DNA sequence is specifically responsible for thyroid hormone responsiveness to a heterologous gene. The catalytic α-subunit of Na^+/K^+-ATPase is also subjected to isoform switching (from α_3 to α_2) during postnatal development. Developmental or physiological stimuli, other than thyroid hormone, regulate Na^+/K^+-ATPase isoform gene expression[33,36]. Another prominent feature of developing heart is the overall increase in metabolic capacity and the shift from carbohydrate to fatty acid utilization[37] and selective upregulation of mRNA encoding enzymes involved in fatty acid metabolism and oxidation[38,39].

The most striking feature of the postnatal cardiac development is the loss of the proliferative capacity of cardiac myocytes[40]. In this respect, a marked distinction can be made between skeletal and cardiac muscle cell development. Muscle specific contractile protein isoforms are expressed only after cell cycle withdrawal in skeletal muscle cell lineage. On the other hand fetal cardiomyocytes express muscle markers during cell proliferation[41]. Furthermore, in response to an increase in workload skeletal muscle is able to recruit precursor cells (satellite cells), whereas heart responds solely by increasing the volume of pre-existing cardiomyocytes. Although, MyoD[42–44], Rb gene[44], Id gene[33], proto-oncogenes and peptide growth factors[45], have been recently implicated to arrest cell cycle in terminally differentiated cardiomyocytes, the exact molecular mechanism is still a major challenge to modern biology.

Cardiac contractile protein genes

The sarcomere is the basic contractile unit of the myocardium[46]. The contractile properties of the myocardium in terms of force generated and velocity of contraction are dependent on the number as well as the biochemical composition of its sarcomeres. The sarcomere is made up of seven major and several minor proteins organized into thick and thin filaments (Table 1). The thin filaments are formed by a double helix of polymerized sarcomeric actin molecules. In the major groove of this double helix, a continuous coil of tropomyosin (Tm) dimers are located (Figure 1). Every tropomyosin dimer interacts with seven actins and it is associated with a troponin (Tn) complex. Each complex in turn is composed of one molecule of each of the three troponins – T, C and I. This tropomyosin–troponin complex regulates calcium sensitivity of the apparatus and interaction between the heads of the myosin molecule, located in the thick filament and actin. The thick filament contains a bifunctional molecule of MHC which consists of rod portion and head portion. The rod portion carries the load during contraction and head portion interacts with actin and carries ATPase activity for fibre shortening. The ATPase activity is modulated by the alkali and regulatory MLC. Fibers with a high ATPase MHC activity contract faster than those with a lower enzymatic activity[47,48]. Thus, the type of MHC and its ATPase activity present in the sarcomeres are physiologically significant.

Table 1. Expression of multiple isoforms of cardiac contractile protein genes

Family	Embryonic isoforms	Adult isoforms	
		Ventricle	Atria
Actin	α-Skeletal α-Cardiac α-Smooth	α-Cardiac	α-Cardiac
Myosin heavy ch.	α-MHC β-MHC	α-MHC	α-MHC
Alkali MLC	MLC-$1_{A\&v}$ MLC-3	$MLCI_v$	$MLCI_A$
Regulatory MLC	MLC-$2_{A\&v}$	MLC-2_v	MLC-2_A
Troponin T	Tn-T_c	Tn-T_A	Tn-T_c
Troponin C	Tn-C_c	TN-C_c	Tn-C_c
Troponin I	Tn-I_{sc}	TN-I_{sc}	Tn-I_{sc}
Tropomyosin	α-Tm β-Tm	α-Tm	α-Tm
Creatine Kinase	CK-BB CK-MM	CK-MM	

MHC, Myosin heavy chain; MLC, Myosin light chain; Tm, Tropomyosin; Tn, Troponin. The subscripts cardiac (c), slow cardiac (sc), ventricular (v), atrial (A) indicate the tissue and or developmental stages in which the gene product is predominantly expressed. Creatine Kinase B and M isoform indicate the muscle-specific (M) and non-muscle (B) isoforms. (Reproduced from Reddy *et al.*, *Indian J. Pharmacol.*, 1996, **28**, 58–71).

The regulated expression of cell type-specific protein isoforms that are structurally distinct and developmentally regulated is a prominent feature of the myocardium. Most of the contractile protein genes that are expressed in the heart[46,49,50] have multiple isoforms (Table 1). Moreover ventricular and conduction system isoforms of the same protein differ from each other[51]. The developmental regulation of isoform expression in embryonic and adult stage is well orchestrated[52]. The physiological advantage of selective isoform distribution is not yet clear.

Regulation of cardiac specific gene expression

Evolution has provided the cardiovascular system with several strategies for the acquisition of tissue-specific phenotypes. Many contractile proteins are encoded by small multigene families whose differential expression in ventricular, atrial and conduction system distinguishes one type of myogenic tissue from the other[53,54]. Understanding of molecular mechanisms that control the cardiac

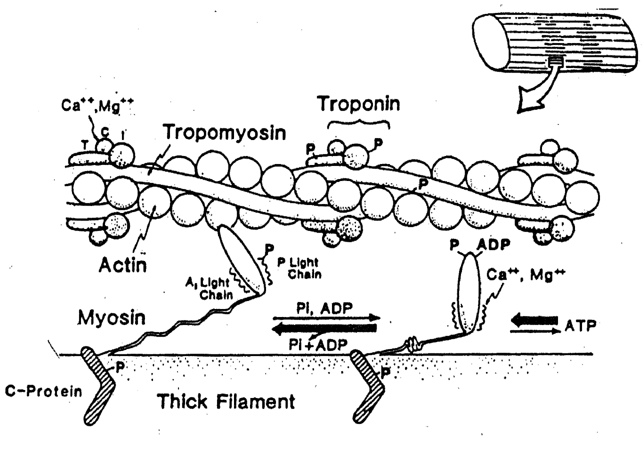

Figure 1. Ultrastructural organization of a sarcomere of cardiac muscle.

muscle specific gene expression is just a beginning, in spite of the limitation afforded by the adult cardiac myocyte with respect to its inability to proliferate and maintain its differentiated phenotype in culture[13]. Moreover, the similarities in morphology and biochemistry of cardiac and skeletal muscle give an idea that tissue-specific expression in these two striated muscle types might follow similar paradigm[41,55].

In recent years complementary DNA (cDNA) and genes encoding many of the contractile elements that are important for the developmental regulation of these genes has revealed the contribution of both transcriptional and post-transcriptional processes for their expression. The nuclei are induced to produce their own muscle regulatory factors which in turn contribute to the modulation of expression of the muscle genes[56]. These protein factors, which are involved in muscle differentiation, are called '*trans*-acting factors', which may be either positive or negative regulatory types[57,58]. Hence, *trans*-acting factors can serve as developmental switches to turn specific genes 'on' or 'off' at appropriate locations and at different times during cardiac development. DNA sequence analysis of muscle-specific genes has allowed the recognition of some sequences of regulatory region important in gene transcriptional regulation. These DNA elements are named as '*cis*-acting sequences' because they are located on the same molecule of DNA as the gene being regulated[59-61]. Some of the *cis*-acting sequences equipped for the high level tissue-specific expression of the cardiac contractile protein genes which utilizes a variety of paradigms for the regulation of gene expression are listed in Table 2.

Recent development of transgenic, transfection and microinjection techniques enabled us to characterize the molecular pathways that control the transcription of muscle genes during cardiac development. Two molecular paradigms for the control of cardiac muscle-specific expression have been recently elucidated[61-68]. Creatine kinase, cardiac and skeletal alpha-actin genes showed E-box-dependent[41,67], and MLC-2, Troponin T,C and MHC genes demonstrated E-box-independent pathways[23,66,68].

Table 2. The *trans*-acting factors and *cis*-acting elements involved in regulation of cardiac muscle specific gene transcription

Trans-acting factor	*Cis*-acting sequence	Gene
MyoD family (ref. 62)	E-Box	α-card. actin α-sk. actin
E Proteins (ref. 63)	E-Box	α-sk. actin α-card. actin
HF 1a (ref. 22)	HF-1	MLC-2 Tn-T_c
HF 1b/MEF-2 (ref. 23)	HF-1	MLC-1 Tn-T_c
AP 5/GTH	AP-5	β-MHC
CBF (ref. 64)	CArG Box	β-MHC α-sk. actin α-card. actin
MCAT F (ref. 64)	MCAT	Tn-T_c
Id (ref. 66)	E-Box (-)	α-card. actin α-sk. actin

E-box dependent pathways

Recent studies of skeletal myogenes have uncovered a number of master transcriptional factors that can dominantly specify the differentiated muscle cell phenotype following their expression in permissive cell types[69-74]. The identification of a host of myogenic determination genes (*MyoD*; *myf-5*; *myf-6/MRF-4/herculin*), has led to a wealth of information on the mechanisms that regulate muscle genes during skeletal myogenesis. MyoD and its family members are expressed both in skeletal and cardiac muscles[41]. The characteristic feature of these transcription factors is that when injected, they can convert fibroblasts into myoblasts[58].

These proteins share a common structural motif, referred to as the basic helix–loop–helix (bHLH) region. The bHLH motif consists of two highly conserved alpha-helices connected by a more variable loop region (Figure 2). This HLH region is preceded by a region which is involved in DNA binding[75]. To activate the muscle gene programme these muscle-specific proteins form heterodimers with other bHLH proteins through interaction of their HLH regions[63,76]. Ubiquitously expressed bHLH proteins E12 and E47, which are splice variants of the E2 gene, provide functionally important dimerization partners of the *MyoD*-like bHLH proteins[76,77]. The *MyoD/E12* heterodimer has a high affinity for the DNA sequence CANNTG (also called E-box site), which is present in the regulatory regions of many muscle-specific genes[77]. The binding of the *MyoD/E12* heterodimer to the E-box site will upregulate the transcription of proteins like muscle creatine kinase[78], MLC-1[79] and cardiac alpha-actin[67]. Another ubiquitously expressed HLH protein, known as Id (inhibitor of differentiation) is also able to interact with *MyoD*. However, this protein lacks the basic DNA-binding region[66], therefore, interaction between *MyoD* and *Id* prevents the formation of a functional heterodimer which can bind to the E-box site. Consequently, activation of muscle-specific genes depends on the cellular levels of *MyoD*, *E12* and *Id*. High level of *Id* prevents the formation of functional *MyoD/E12* heterodimer complexes. Overexpression of Id down-regulates transcription from both skeletal and cardiac alpha-actin promoter–reporter constructs in myocytes[80] and mutation in E-box sites negatively affect the expression of genes[67]. The role of E-box-dependent pathways in the control of cardiac gene programme is still not settled.

E-box independent pathways

Recent studies have led to the characterization of the *cis*-regulatory elements and their corresponding factors, which are components of the E-box-independent pathways for cardiac muscle-specific expression[41]. This A + T rich DNA sequences have been found in the promoter/enhancer regions of various muscle genes such as creatine kinase[81], cardiac troponin T (ref. 65), and MLC-2 (refs 22, 82). Sequence comparison of rat MLC-2 promoter revealed three highly conserved regions (*HF-1*, *HF-2*, *HF-3*) within the first 250 base pairs of the promoter[82]. Transient transfection studies have shown that this region was sufficient to confer cardiac-specific expression. Transgenic studies[83], gel mobility shift assays and methylation interference analysis[22] demonstrated that two nuclear proteins specifically bind to the *HF-1* site. One designated as HF-1a is found to bind the core region of the 28 base pair of *HF-1* element (Figure 2). The second, HF-1b binds to an A + T-rich region which is homologous to the so-called myocyte enhancer factor-2 (*MEF-2*) binding site in the muscle creatine kinase enhancer[23]. Transient transfection assays have indicated that both HF-1a and HF-1b binding regions are required for transcriptional activity in cardiac muscle cells. Recently, a HF-1b binding protein factor is isolated from a cDNA library from neonatal rat hearts[24] and it represents a novel zinc finger protein with homology to *SP-1* (Figure 2). HF-1b is expressed in both skeletal and cardiac muscles. Unlike E-box sites, A+T rich sites have been shown to be important in the regulation of gene transcriptions in myocardium[54]. The elucidation of the precise molecular mechanisms that control cardiac specific gene expression will depend upon the cloning, characterization and functional analysis of factors which confer tissue specificity.

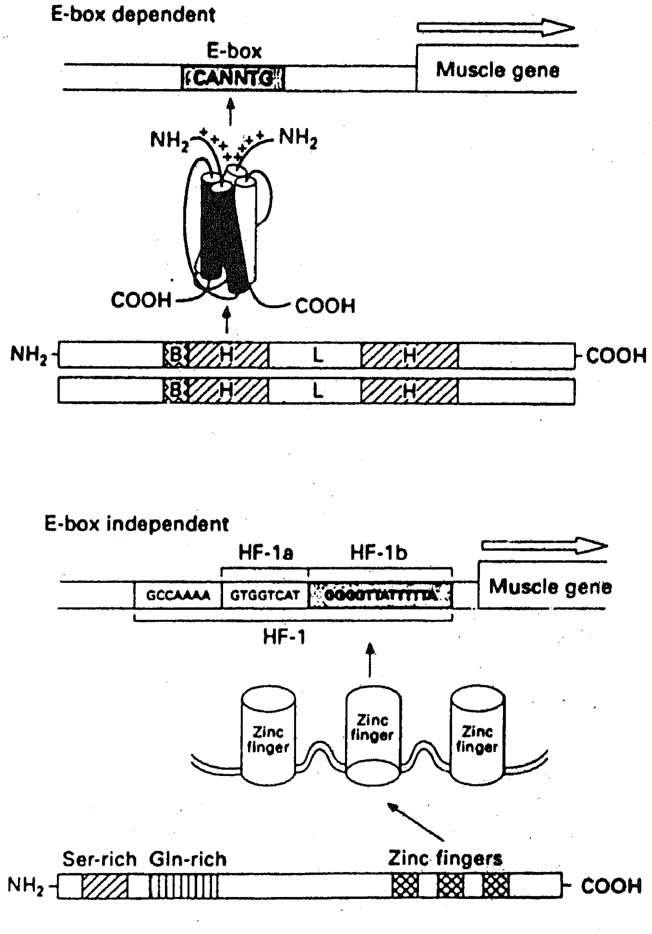

Figure 2. The *trans*-acting factors and *cis*-acting elements involved in E-box dependent and E-box independent regulation of cardiac muscle gene transcription[13].

Pathobiology of cardiac hypertrophy

It is now apparent that hypertrophy of the overloaded heart is a complex process that is both beneficial to compensate the overload, and detrimental because the hypertrophied cells are not normal[84]. Since the myocytes are terminally differentiated and lose their ability to proliferate soon after birth, they respond to increase in workload by an increase in cell size (hypertrophy), and not by an increase in cell number (hyperplasia). Chronic increase in wall tension lead to left ventricular hypertrophy, ventricular dysfunction (Figure 3) and ultimately

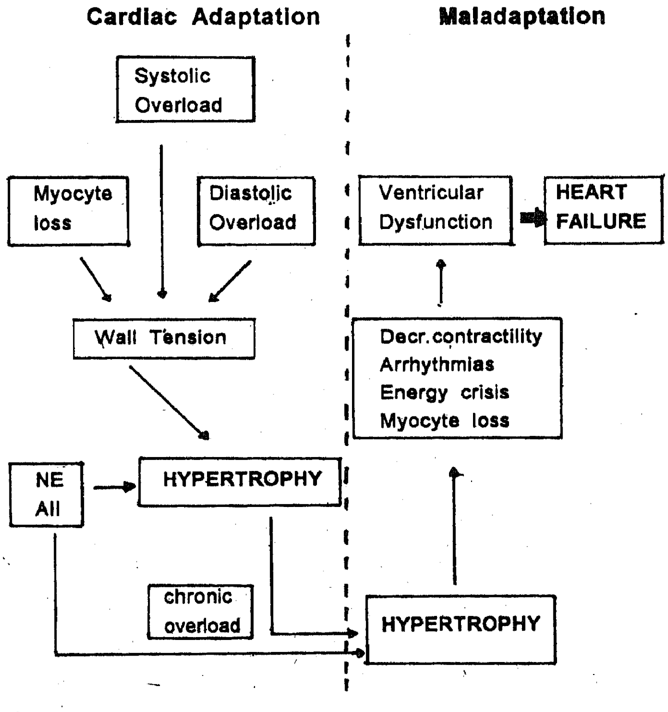

Figure 3. Postulated pathophysiological scheme of cardiac hypertrophy and failure.

decompensated hypertrophic heart failure if adequate measures are not taken[85]. The extent of hypertrophy is also modulated by the release of autocrine or paracrine factors such as norepinephrine, angiotensin-II, and peptide growth factors. In various models of cardiac hypertrophy, systolic and diastolic wall stress have been implicated as the major determinants of the degree and patterns of hypertrophy during pressure and volume overload[86].

Morphological changes

The structural and functional abnormalities of the hypertrophied myocardium have been examined in depth. An increase in the load against which the heart ejects, gives rise to adaptive changes in the heart which vary with the species studied and severity of the overload. The myocytes become bigger, contract and relax more slowly as a consequence of an adaptive mechanism[87]. In addition, ventricles become stiff and premature beats are more frequent[84]. Increase in cardiocyte size[88] and interstitial connective tissue[89,90] may be responsible for the stiffness of hypertrophied myocardium. The walls of the heart grow thick initially in response to overload[91], but become thin in end-stage of decompensated hypertrophy. Myocyte necrosis stimulates proliferation of the fibroblasts replacing myocardial cells with connective tissue[92]. Progressive overload on the surviving cells of the hypertrophied heart, relative decrease in capillary density and number of mitochondria probably contribute to a chronic energy deficit. The growth of the coronary vascular may not commensurate with increased cardiac mass and work load accompanying cardiac hypertrophy[93] especially in the subendocardial layer[94] and may thus lead to episodic underperfusion and myocardial damage. Alterations in muscle chamber compliance during hypertrophy have been identified as a result of potential decrease in contractile function[86]. Increased capacity for and efficiency of protein synthesis may account for rapid cardiac growth[95,96] and change in gene programme may lead to variations in electrophysiological and biochemical properties of hypertrophied heart.

Electrophysiological changes

Action potential duration (APD) is prolonged in isolated multicellular[97,98] and single cell preparations of hypertrophied hearts[99,100]. Such a change may be compensatory response to ensure maximal contraction by slowing V-max, but it impairs cardiac relaxation via myoplasmic calcium overload[101]. Prolonged APD may thus be linked to the contractile abnormalities found in hypertrophy[102] and high incidence of arrhythmias. An increase in cell capacitance consequent to enhanced surface area of myocytes does not account for the observed prolongation of APD. Changes in the current-carrying system of the sarcolemma may explain the noted increase in APD. Increased APD may contribute towards the increase in contractile strength and cardiac output of hypertrophied heart[103].

Biochemical changes

The rate of energy utilization is abnormally increased in acute pressure overload hypertrophy[104], resulting in energy crisis. The myosin ATPase activity, associated with energy utilization is decreased as a result of an isoenzymatic shift from V_1-(high ATPase activity) to the V_3-(low ATPase activity) of myosin[105]. This adaptation may exercise economy in the use of energy in hypertrophied myocardium. β_1-Adrenergic receptors are down-regulated in spite of normal noradrenaline levels[106], but α_1-adrenergic receptor population markedly increased in cardiac hypertrophy[107]. Cholinergic muscarinic receptors are also found down-regulated. The increase in calcium channel number[108], decrease in activity of Na^+/Ca^{2+}-exchanger[109], α-subunit isoform shift of Na^+/K^+-ATPase (ref. 110), diminished function of Ca^{2+}-ATPase of sarcoplasmic reticulum[111], and down regulation of β_1-adrenergic receptors are postulated to lead to disturbance in calcium homeostasis and ventricular arrhythmias.

Gene expression changes in hypertrophied myocardium

Application of recombinant DNA technologies and cellular biological methods during the last 10 years to cardiac myocytes enabled us to understand that the cardiac phenotype changes with ontogeny, aging and hypertrophy[50]. The ability of the heart to alter its protein composition is a tonic control mechanism of cardiac function. The heart first responds to increased workload by altered function, followed by gene expression and increased muscle mass. Hemodynamic overload produces a complex pattern of gene programming characterized by changes in protein isoform switches and quantitative alteration in individual gene expression in the myocyte[50,112].

Recently, many genes responsive to increased workload have been identified and are divided into two classes[10]: one designated as immediate early genes (IEG), and the second, late response genes (LRG). IEGs are a group of proto-oncogenes whose transcription is activated rapidly and transiently by extracellular stimulation[113-116]. The transcriptional induction is not dependent on new protein synthesis, but subsequent shut-off of transcription requires new protein synthesis[117,118]. The expression of LRG is induced more slowly, over hours, and also needs new protein synthesis. LRG expression includes, upregulation of constitutively expressed cardiac contractile protein genes and re-expression of fetal-type genes.

Expression of proto-oncogenes

One of the early responses of cardiac cells to overload is the induction of a series of proto-oncogenes[115], that directly or indirectly turn on the cascade of events leading to hypertrophy. Proto-oncogenes owe their name to the fact that they are cellular counterparts of viral oncogenes and altered genes of neoplastic cells[118]. However, recently it has become clear that proto- oncogenes or cellular oncogenes (*c-onc*) are bonafide transcriptional factors which participate in normal growth induction of the cell in response to growth stimuli[119]. Many proto-oncogenes control the re-entry of resting cells (G_0 phase) into the cell division and regulate the transcription of a variety of genes. Proto-oncogenes exert their regulatory effects by encoding a variety of growth factors and their receptors, intracellular transducers, modulators, amplifiers, and DNA-binding factors involved in the control of RNA transcription[120,121]. Approximately 100 IEGs have been identified so far[10]. Several proto-oncogenes of the *fos* family such as *c-fos, fra-1 fos-B* (refs 122–124), *jun* family such as *c-jun, jun B, jun D* (refs 125, 126), *c-myc* (ref. 127), *Egr-1* family such as *krox 24, NGF1-A* (refs 128–131), and *nur/77* or *NGF1-B* (refs 128, 132) have been characterized.

Proto-oncogenes such as *c-fos* and *c-myc* are induced *in vivo* and *in vitro* rat heart models[115,133-136]. The expression levels of proto-oncogenes is proportional to the magnitude of coronary flow, coronary pressure and systolic wall stress[137]. Stretching of myocytes by *in vitro* model[116,138–140] stimulates the expression of *c-fos*, *c-myc*, *c-jun* and *Egr-1*, in a stretch length-dependent manner followed by increase in protein synthesis. Hemodynamic overload increases the expression of *c-fos* and *c-myc* proto-oncogenes that encode short-lived nuclear proteins that promote and regulate cell proliferation and differentiation[115,120,141,142]. The activation of these proto-oncogenes may be an adaptive response to stress. The induction of *c-fos* and *c-myc* which occurs within an hour of acute pressure overload, is transient and precedes the expression of fetal isoforms of several contractile protein genes[115].

Recent studies revealed that the protein products of *c-fos* and *c-jun* interact with each other to form a heterodimeric transcription factor complex through a conserved dimerization motif referred to as leucine zipper[143]. The leucine zipper domain is an alpha-helical structure composed of leucine residues spaced seven amino acids apart and promotes dimerization. The *c-fos/c-jun* heterodimer binds with high affinity and specificity to DNA elements of the consensus sequence called TRE. TRE is first identified as phorbal ester-inducible promoter element and is a binding site for transcription factor *AP-1* (refs 102, 144), *c-fos* and *c-jun* are major constituents of *AP-11* (ref. 145). The expression of *c-fos* and *c-jun* is induced rapidly by a number of external stimuli and consequently *c-fos/c-jun* complex induces the expression of LRG containing *AP-1* sites[146]. *c-fos/c- jun* complexes transactivate a number of genes containing *AP-1* sites, where *c-fos/jun B* complexes repress them under certain circumstances[147]. IEGs also have a regulatory function in post-mitotic cells[113].

The mechanisms by which proto-oncogenes are induced are best characterized by studies of *c-fos*. *c-fos* is induced by multiple intracellular second messengers acting through distinct upstream regulating elements. Transcriptional activation by PKC is mediated by serum response element (SRE) located 300 bp upstream of the mRNA initiation site. The SRE consists of 20 bp dyad symmetric sequence and is a specific binding site for dimeric SRF and other associated factors such as *SAP-1* (refs 148, 149). *c-fos* stimulation by cAMP and Ca^{2+} is dependent on Ca^{2+}/cAMP regulatory element (CRE) located 60 bp upstream of the mRNA start site. The Ca^{2+}/CRE is an 8 bp palindromic sequence and is a specific binding site for the dimeric transcription factor (CREB). The increase in intracellular cAMP and Ca^{2+} activates protein kinase A and Ca^{2+}/calmodulin kinase, respectively, and these kinases phosporylate CREB at a specific residue (ser-133) in order to activate it as a transcription factor[150].

Cellular proliferation and differentiation is regulated by *c-myc* (ref. 151). Increased expression of *c-myc* results in an increase in the rate of proliferation and prevents differentiation[152,153]. Inhibition of *c-myc* expression by antisense strategy inhibits proliferation and initiates differentiation of variety of cell types[154]. *c-myc*, as a transcription factor induces a family of heat shock proteins[155]. Recently, *Max* a partner of *c-myc* in forming a heterodimer complex has been isolated, and the transcriptional functions of *c-myc* have been further elucidated[156]. *Egr-1* is also isolated as IEG to growth factors. Atrial natriuretic factor[157] and transforming growth factor-β[158] have *TRE/AP-1* binding sites in the promoter regions and may become targets of these proto-oncogenes. Recently using co-transfection assays it has been shown that *c-jun* or *c-jun/c-fos* transactivate human skeletal alpha-actin gene expression, suggesting that skeletal alpha-actin may be regulated by *c-fos/c-jun* complex in addition to *AP-1/TRE* mechanisms. Other transcriptional mechanisms activated by the *c-fos\c-jun* complex may also be present[159]. *c-myc* is abundantly expressed in the heart during the fetal period; however its expression becomes undetectable soon after birth[115]. Elevated *c-myc* mRNA expression stimulates hyperplastic myocyte growth, but it does not induce cell hypertrophy and alterations of terminal differentiation[160]. *Egr-1* is induced by hemodynamic overload, alpha-adrenergic receptor stimulation and stretch of myocyte[161]. It has a zinc finger DNA-binding motif and the ability to transactivate other genes which have the target sequence C G C C C C G C (ref. 162). *Egr-1* may also transactivate some cardiac genes such as alpha-MHC as a result mechanical stress[163].

Different stimuli induce the expression of slightly different panels of proto-oncogenes, and are associated with different hypertrophic phenotypes of the cardiomyocyte. Pressure overload leads to a form of hypertrophy that is characterized by a reduction in sarcoplasmic reticular Ca^{2+}-ATPase activity[164–166] and a shift from α-MHC to β-MHC isoform expression. In contrast, thyroid hormone induces a different form of hypertrophy that is associated with increased Ca^{2+}-ATPase activity[165,167] and enhanced α-MHC isoform expression. Stimulation with α- and β-adrenergic receptor agonists is also associated with different expression of IEGs[161]. These evidences suggest that proto-oncogenes might control the expression of late response genes as nuclear mediators that couple external stimuli to long-term changes in gene expression.

Re-expression of the fetal isogenes

The major contractile proteins of the heart have multiple isoforms (Table 1). Tropomyosin, Tn-T and MLC isoforms are generated by alternative pre-mRNA splicing[168], and isoforms of MHC, MLC, actin, tropomyosin, Tn-T, Tn-I and Tn-C are produced by a multigene family[46]. The expression of each member of this multigene family is regulated tissue specifically at the transcriptional level. In cardiac hypertrophy, the contractile protein genes are selectively activated either by pre-mRNA splicing[141] or at transcriptional level[169,170]. Gene phenotypes of contractile proteins such as skeletal alpha-actin[171], MLC-1 (ref. 172), tropomyosin[141] and troponins[173,174], membrane proteins such as Na^+/K^+-ATPase[175–177], and those involved in ATP regeneration such as creatine kinase[178] are induced in cardiac hypertrophy. Most of these protein isoforms are normally expressed only during fetal and early post-natal life (Table 3).

Myosin heavy chain (MHC), a late response fetal gene is investigated at the physiological and molecular levels[105]. In the ventricles of most mammalian species three myosin isoforms have been identified on the basis of electrophoretic mobility – V_1, V_2, V_3 (ref. 179). However, these three myosins are composed of only two distinct types of MHCs, referred as alpha and beta. V_1 and V_3 are composed of alpha–alpha and beta–beta homodimers, respectively, while V_2 is an alpha–beta heterodimer. These two myosins are produced by two different genes[180]. The relative distribution of α- and β-MHC is directly correlated with the contractile properties of the heart. The α-MHC, which has high Ca^{2+} and actin-activated ATPase activity, is associated with an increased muscle-shortening velocity. In contrast, the β-MHC, which has lower ATPase activity is associated with slower shortening velocity[47,105].

Pressure overload hypertrophy induces a qualitative change in myosin composition from alpha-(V_1) to beta-(V_3)-isoform[164]. This alteration in myosin composition is largely due to down regulation of the alpha-MHC gene and concomitant re-activation of β-MHC gene expression[32]. The reactivation of β-MHC gene is one of the first well-characterized molecular markers for the activation of an embryonic gene programme during myocardial hypertrophy[181,182]. The change from α-MHC to β-MHC results in slower rate of ATP cycling by myosin which accounts for slower velocity of contraction of hypertrophied heart[183]. However the tension generated during each systole is increased which facilitates ejection by the overloaded heart. Furthermore, sliding of actin and myosin filaments slows down, leading to minimal energy consumption. This sequence of events is considered to be an adaptive response of heart to the mechanical overload. The *cis*-regulatory elements and corresponding *trans*-acting factors mediate the up-regulation of the beta-MHC genes during hypertrophy[184,185]. Kariya *et al.*[135,136] have localized an α_1-adrenergic responsive fragment within the β-MHC promoter region that mediates expression to α-adrenergic stimulation in myocardial cell hypertrophy. Ventricular hypertrophy in larger animals and humans is not associated with the induction of either β-MHC or actin genes, which are constitutively expressed in large animals[182,184]. However in human atria, change of α-MHC to β-MHC is readily apparent in response to increased pressure overload[186]. In contrast,

Table 3. Phenotypic changes in myocardial ventricular proteins during cardiac hypertrophy

	Fetal	Adult	Hypertrophy
MHC	β-MHC α-MHC	α-MHC	β-MHC
α-Actin	skeletal cardiac	cardiac	cardiac
MLC-1	MLC-1_v MLC-1_A	MLC-1_v	MLC-1_v MLC-1_A
MLC-2	MLC-2_{v+}	MLC-2_{v+}	MLC-2_{v+}
Tropomyosin	α-Tm β-Tm	α-Tm	α-Tm β-Tm
Na^+/K^+-ATPase	α_1, α_2	α_1	α_1, α_2
ANF	+	–	++
SR Ca^+-ATPase	+	++	+

+, Normal; ++, Increase/upregulation.
(Reproduced from Reddy *et al.*, *Indian J. Pharmacol.*, 1996, **28**, 58–71.)

a marked increase in the skeletal α-actin mRNA has been reported in a diseased human heart[187].

Skeletal α-actin gene, another marker of the embryonic gene programme, is uniformly induced throughout the ventricles[188]. The *cis*-regulatory elements and corresponding *trans*-acting factors that mediate upregulation of skeletal α-actin[189] and smooth muscle actin[170] during the hypertrophy are incompletely characterized. The genes encoding MHC isoforms are regulated in an antithetical manner in early stages of hemodynamic overloads, whereas hemodynamic overload up-regulates skeletal alpha-actin gene but does not affect the cardiac alpha-actin[190]. Thus the different sarcomeric components in the same heart are not equivalent targets for the mechanisms activated by changes in external cardiac load in hypertrophy. A 67 bp fragment in skeletal actin gene conferred myocyte specificity and inducible ability by alpha-1-adrenoceptor stimulation[191]. It contains a CArG box, SP1 and M-CAT sequences. Knowlton *et al.*[192] determined *cis*-elements that mediate alpha-adrenergic expression within 315 bp sequence of ANF (*CRE*, *AP-2*, *AP-1*, *Egr-1*, CArG) and 92 bp of MLC-2 (*AP-1*, *CArG*, *MEF-2*, *AP-2*) gene.

ANF is expressed in the ventricle as well as in the atrium during embryonic development but after birth the expression of ANF is restricted to the atrium. However being a potent vasodilator and natriuretic agent, ANF is abundantly re-expressed in ventricles subjected to overload in order to reduce wall stress[177]. ANF gene is utilized as a marker for embryonic gene programme during hypertrophy[15]. A variety of hypertrophic stimuli induces ANF gene in ventricular muscle cells[61] and therefore is a useful genetic marker of cardiac hypertrophy. Regional-specific expression of ANF in the atria[193] is clearly segregated from those that are responsible for inducibility[194]. Recent studies have mapped the inducible regulatory elements in the rat ANF gene to a proximal region of ANF promoter that lacks consensus *Egr-1*, *CRE*, *AP-1*, *CArG* element. ANF gene expression is regulated by a variety of stimuli such as hormones[177,195], and physical stretch of myocardial tissue[176]. Activation of PKC induces secretion of ANF as well as expression of ANF gene[196]. PKC activates the *c-fos* and *c-jun* genes. *c-fos/c-jun* complex interacts with *AP-1* binding sites and activates the ANF gene expression suggesting the potential role of proto-oncogenes in ANF gene expression in hypertrophy.

Genes encoding Ca^{2+}-ATPase of the sarcoplasmic reticulum are altered by hemodynamic overload[197]. The mRNA level of Ca^{2+}-ATPase decreases gradually in pressure overload[165,198] and expression levels are reduced in the failing human heart[166]. The decrease in the Ca^{2+}-ATPase protein may be accounted for the abnormal calcium handling in the hypertrophic and failing hearts.

Induction of constitutive gene programme

In addition to qualitative alterations in the cardiac muscle gene programme, there are quantitative alterations in the expression of constitutively expressed contractile protein genes that lead to characteristic accumulation of contractile proteins and sarcomeric units in individual cardiac muscle cells. MLC-2 gene investigated extensively[22,192,199] was found up regulated. Recent studies in both cultured myocardial cell models[192] and pressure overload transgenic mice which harbour a 250 bp MLC-2 luciferase fusion gene[83] revealed that the 250 bp fragment which confers cardiac-specific expression is also sufficient for inducible expression. Mapping studies of the MLC-2 gene documented an important role for the *HF-1* for cardiac specific and inducible expression to heterologous promoters[22]. The recent development of an *in vivo* murine model of cardiac hypertrophy[200] that can be used in transgenic approaches in myocardial hypertrophy model systems[201–203] may enable us to characterize genetic markers and novel factors of gene expression in hypertrophy.

Role of humoral factors and signalling mechanisms

Although the phenomenon of cardiac hypertrophy has been extensively investigated, the mechanisms that initiate cardiac cellular growth, change in myocardial gene expression in response to work overload and cellular signalling melecules remain incompletely understood. The development of cytoplasmic injection of neutralizing antibodies[204] and purified recombinant proteins as well as nuclear injection of expression vectors in contracting cardiac muscle cells[203] has provided a valuable tool to distinguish between cellular signalling messengers from hypertrophic phenotype markers. Recent studies on hypertrophic phenotype markers in cultured myocardial cell model[205], enabled us to unfold the potential cellular signalling pathways responsible for cardiac growth and hypertrophy (see Figure 8).

Mechanical stretch

Mechanical stretch of the ventricular wall is associated with increased protein synthesis[206,207] and activation of a number of hypertrophic markers[208–214]. Effect of ventricular stretch on growth is a cAMP dependent mechanism[215]. Myocyte stretching stimulates expression of proto-oncogenes, followed by expression of fetal genes[116,138–140]. Stretch-activated ion channels have been proposed as the transduction mechanism between load and protein synthesis in cardiac hypertrophy[216]. Stretch-sensitive channels which allow the passage of physiological cations, such as Na^+ and K^+, and Ca^{2+} are implicated[216-218]. Na^+ influx is demonstrated to increase protein synthesis in heart. However Na^+ ionophores such as monensin and veratridine did not reduce fetal type gene expression in cultured cardiomyocytes[218]. Cardiomyocytes, endothelial cells, fibroblasts and smooth muscle cells may secrete some hypertrophy-promoting factors following a stretch stimulus.

Myotrophin and peptide factors

A cardiac hypertrophy-related factor 'myotrophin' has been demonstrated to exist in the hypertrophied myocardium of spontaneously hypertensive rats[219]. It has been purified[219] and its nature and physiological roles are being explored. Myotrophin stimulates protein synthesis *in vitro* in both neonatal and adult cardiac myocytes[219], and it has also been shown that incubation of neonatal rat cardiac myocytes with 'myotrophin' for 30 min induces proto-oncogenes like *c-myc*, *c-fos* and *c-jun* analogous to the pressure overload- induced expression of proto-oncogenes. Further, increased accumulation of mRNA for ANF, β-MHC and skeletal α-actin have been observed in cardiomyocytes treated with this protein for 24 h (ref. 220), suggesting that the 'myotrophin' may be a pivotal modulator for myocardial cell growth and differentiation.

Numerous peptide factors have also been reported to be elevated in the plasma of patients with cardiac disorders. A 17.8 kDa hemo-protein has been demonstrated in the serum of patients with myocardial infarction[221]. Increased level of a hypertension associated protein of molecular weight 28 kDa has been observed in animals and humans with essential hypertension[222,223]. Presence of a 182 kDa protein has been found 6 h after the aortic constriction in animals, and persisted during the progression of hypertrophy and disappeared at late stages[224,225]. Multiple injections of polyclonal antibody raised against this protein into the aorta constricted animals totally abolished the development of cardiac hypertrophy and also suppressed the expression of β-MHC. Moreover, elevated levels of this peptide have been reported in patients with ventricular septal defect and aortic regurgitation further suggest that this peptide factor could play a role in the development of hypertrophy and the molecular mechanisms associated with cardiac hypertrophy[226,227].

Renin–angiotensin system

The renin–angiotensin system (RAS) is integrally involved in cardiovascular and renal homeostasis[228]. The principal mediator of the physiological actions of the RAS is the octapeptide angiotensin-II (A-II). Angiotensinogen, the precursor of A-II is hydrolysed by the enzyme renin, thus resulting in the formation of the decapeptide angiotensin-I (A-I). The two carboxyterminal amino acids of A-I are cleaved by angiotensin converting enzyme (ACE) to form the octapeptide A-II (ref. 229). A-II directly modulates cardiac functions such as myocardial contractility, metabolism and hypertrophic growth[10,230-232].

An increasing number of studies suggested the existence of local renin–angiotensin system in several organs such as the kidney, adrenal gland, brain, blood vessels and heart[3-5,233-236]. Much evidences support the existence, functional integration and physiopathological role of cardiac RAS[1,237-239]. Molecular biological and biochemical techniques clearly demonstrated local synthesis of mRNA of angiotensinogen, renin, and converting enzyme in the heart, indicating the presence of a physiologically active cardiac RAS. A-II has been suggested as an important hormonal mediator of cardiac hypertrophy[238]. It is one of the growing number of peptide hormones that has been implicated in the regulation of cellular growth[240] and hypertrophy[241]. The direct growth effects of A-II have been extensively characterized in vascular smooth muscle cells, where A-II has been shown to promote hypertrophy *in vitro*[242-244]. A-II also causes hyperplasia of aortic smooth muscle cells of spontaneously hypertensive rats and the renal arteriole of normal rats[245,246], suggesting that A-II directly or in combination with other growth factors may play an important role in the development of vascular hypertrophy and elevated arterial resistance in hypertension.

Several *in vivo* studies have suggested that A-II may also be a critical factor in mediating cardiac hypertrophy that is associated with hypertension[247,248]. ACE inhibitors prevent or decrease LVH in animals[249-251] and in humans[252,253]. Clinical studies[254] demonstrated that treatment with ACE inhibitors reduced mortality in patients with heart failure. The ameliorative effects of ACE inhibitors may be ascribed to either hemodynamic changes such as reduction of systemic vascular resistance and LVEDP or alterations of cardiac RAS. Chronic infusions of sub-pressor doses of A-II to rats produced ventricular hypertrophy[254]. In genetic model of hypertension, ACE inhibitor produced regression of cardiac hypertrophy while control of rise in blood pressure with sympatholytic agents or vasodilators demonstrated no such effect[247,251]. Nonhypotensive dose of ramipril prevented the increase of cardiac mass in rats with aortic banding[251]. In addition, enalapril completely prevented the LVH with no effect on cardiac afterload[241]. Beinlich *et al.*[255] have demonstrated that AT_1 receptors may be involved in growth of the newborn pig heart. Treatment of patients suffering from myocardial infarction with ACE inhibitors prevented cardiac dilatation[256]. These observations tentatively suggest, in addition to indirect effects of A-II mediated increase in blood pressure and vascular resistance, that A-II may be directly responsible for the hypertrophy of heart.

ACE inhibitors also prevented the development of age-dependent hypertrophy[257]. A recent meta-analysis of 109 studies showed that ACE inhibitors are more effective than β-blockers, calcium antagonists and diuretics in reducing the left ventricular mass of hypertensive patients[258]. Multivariate analysis of the results showed that the reversal of cardiac hypertrophy is indirectly related to the blockade of RAS[259]. Captopril prevents myocardial fibrosis, collagen accumulation and DNA synthesis in rats with renovascular hypertension[260] and in myocardial infarction[261]. It indicates that A-II may play a role in the structural remodelling of the cardiac interstitium. Moreover the process of interstitial remodelling may lead to LVH and subsequent heart failure[262]. Recently, Schorb *et al.*[263] have shown that A-II is mitogenic to neonatal rat cardiac fibroblasts. Non-myocytes such as cardiac fibroblasts have G-protein-linked AT_1 receptors that are coupled with proliferative growth[264,265] and A-II induced cardiac hypertrophy may be partly due to stimulated increases in nonmyocyte cellular growth. A-II increases protein synthesis, total RNA and *c-fos* mRNA in cultured cardiomyocytes[265,266]. Increased rate of protein synthesis is blocked by a specific AT_1 receptor antagonist[267]. These observations suggest that A-II may induce hypertrophy by enhancing gene expression.

AT_1 and AT_2 receptor expressions are upregulated in response to hypertrophic stimuli[264]. The increased number of A-II receptors may contribute to the growth of heart and hyperplasia of cardiac fibroblasts. A-II induces IEGs such as *c-fos*, *c-jun* and *Egr-1* in cardiac myocytes and nonmyocytes. A-II also induces the 'fetal' genes such as ANF and α-skeletal actin and upregulates the expression of growth factor genes such as TGF-β and angiotensinogen. Activation of multiple phospholipid derived second-messenger systems via the AT_1 receptors in cardiomyocytes[265,268] may play a critical role in A-II induced gene expression and subsequent hypertrophy. Increased cardiac ACE activity[269], upregulation of mRNA of ventricular angiotensinogen[241,270] and mRNA of ACE[269] have been described in association with pressure-overload cardiac hypertrophy. It suggests that cardiac RAS may be activated in cardiac hypertrophy.

Several lines of molecular and biochemical studies from our laboratory have suggested that cardiac RAS plays a pivotal role in the pathogenesis of ventricular hypertrophy[1-4]. Treatment with hypotensive dose (100 mg/kg), as well as non-hypotensive dose (1.5 mg/kg) of captopril, an ACE inhibitor (Figure 4), markedly prevented the development of LVH (Figure 5), LV remodelling and prevented the increase in total ventricular protein, RNA (Figure 6) and antithetical expression of myosin heavy chain isogenes (Figure 7) in cardiac pressure-overloaded rats[1]. However, treatment with hydralazine (15 mg/kg), a vasodilator, did not modulate the hypertrophic changes[1]. Thus, the prevention of the development of LVH and induction of β-MHC by non-hypotensive doses of captopril may be related to the blockade of intracardiac production of angiotensin-II rather than circulating system.

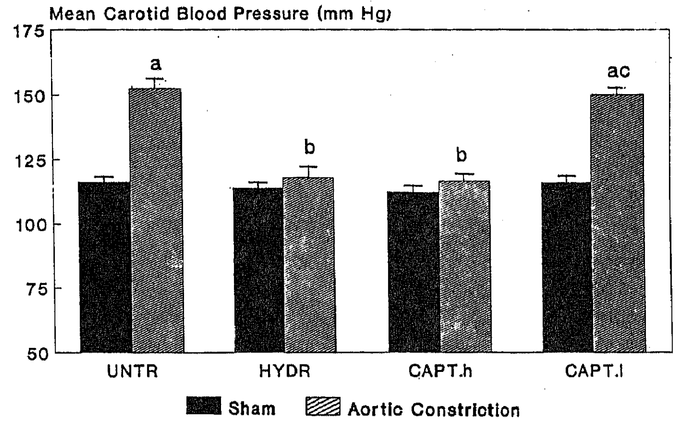

Figure 4. Effects of chronic treatment with captopril or hydralazine on mean carotid blood pressure in response to cardiac pressure overload induced by abdominal aortic constriction. Values are means ± S.E.m. $n = 5$–8 per group. Sham (filled bars); aortic constriction (hatched bars); UNTR, untreated; HYDR, hydralazine (15 mg/kg, p.o.); CAPT.h, captopril-high dose (100 mg/kg, p.o.); CAPT.l, captopril-low dose (1.5 mg/kg, p.o.), a $P < 0.05$ vs sham; b $P < 0.05$ for captopril/hydralazine treated vs untreated group; c $P < 0.05$ for captopril-aortic constriction-treated vs sham captopril-treated group. (Reproduced from Reddy *et al.*, *Mol. Cell. Biochem.*, 1996, **155**, 1–11.)

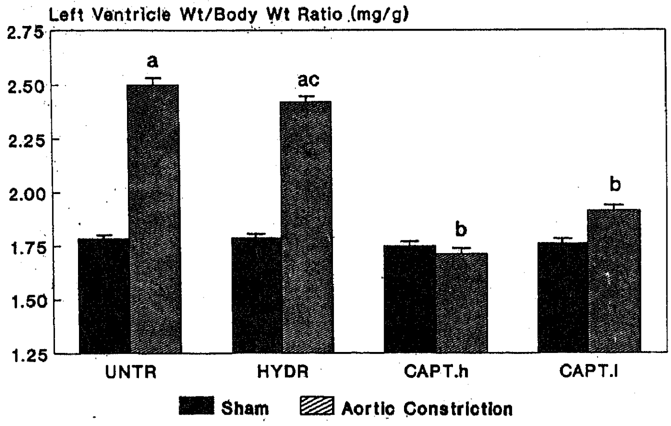

Figure 5. Effects of chronic treatment with captopril or hydralazine on left ventricular hypertrophy in response to cardiac pressure overload induced by abdominal aortic constriction. Values are means ± S.E.m. $n = 5$–8 per group. Sham (filled bars); aortic constriction (hatched bars); UNTR, untreated; HYDR, hydralazine (15 mg/kg, p.o.); CAPT.h, captopril-high dose (100 mg/kg, p.o.); CAPT.l, captopril-low dose (1.5 mg/kg, p.o.), a $P < 0.05$ vs sham; b $P < 0.05$ for captopril/hydralazine treated vs untreated group; c $P < 0.05$ for hydralazine-aortic constriction-treated vs sham hydralazine-treated group. (Reproduced from Reddy *et al.*, *Mol. Cell. Biochem.*, 1996, **155**, 1–11.)

Sympathetic nervous system

For long time, sympathetic nervous system activity has been implicated in development or modulation of cardiac hypertrophy[271,272]. *In vivo* studies also indicated that stimulation of either α- or β-adrenergic receptors leads to the development of myocardial hypertrophy independent of hemodynamic effects[273]. It has been demonstrated that α_1-adrenergic stimulation induces a hypertrophic response in both isolated adult myocytes[266] and neonatal myocytes. But β-adrenergic stimulation of isolated adult myocytes has a more general anabolic effect[107,274–279], and induces limited hypertrophic response. Alpha-1-adrenergic receptor stimulation increases diacyl glyceraldehyde (DAG) and protein kinase C (PKC) activity which may represent a link between receptor binding and accelerated transcription[203,280]. Administration of phorbol ester, a specific activator of PKC to the cultured myocytes leads to cellular hypertrophy[281]. Transient transfection of cells with plasmid encoding constitutive gene of PKC increases hypertrophic response[135,136]. PKC possibly affects gene transcription through phosphorylation of pre-existing transcription factors or through induction of novel transription factors (Figure 8)[282]. Expression of nuclear proto-oncogenes such as *c-fos* has been reported in response to nor-epinephrine[283] and isoproterenol- induced[284] cardiac hypertrophy.

Polypeptide growth factors

Recent studies, both *in vivo* and *in vitro* suggest that myocardium is the target of selected growth factors[285], and may play an important role in both normal and abnormal, such as hyperplastic and hypertrophic growth of heart[286]. The multifunctional polypeptide growth factors (PGF) may regulate the myocardial growth and differentiation. Two classes of growth factors, namely

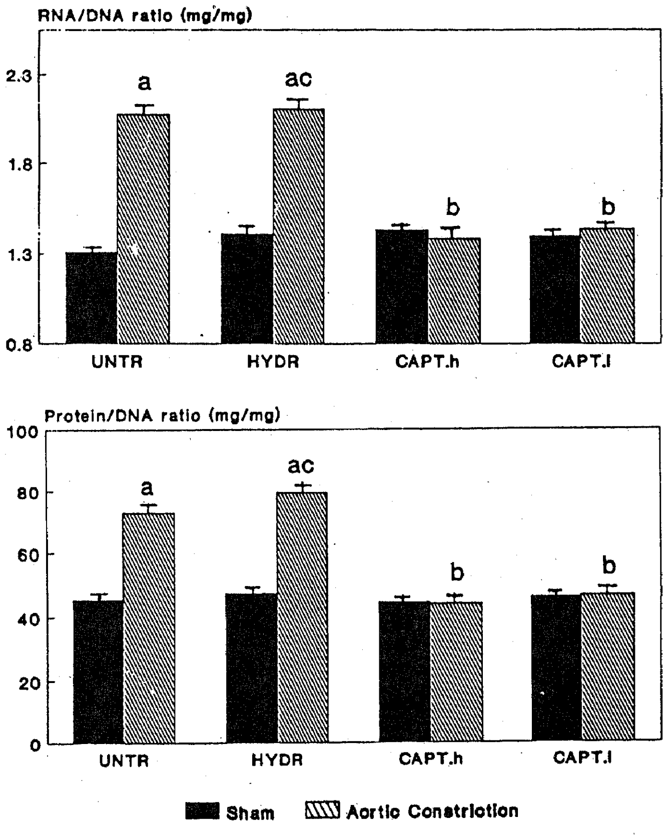

Figure 6. Effects of chronic treatment with captopril or hydralazine on RNA/DNA and protein/DNA ratios in response to cardiac pressure overload induced by abdominal aortic constriction. Values are means ± S.E.m. $n = 5$–8 per group. Sham (filled bars); aortic constriction (hatched bars); UNTR, untreated; HYDR, hydralazine (15 mg/kg, p.o.); CAPT.h, captopril-high dose (100 mg/kg, p.o.); CAPT.l, captopril-low dose (1.5 mg/kg, p.o.), a $P < 0.05$ vs sham; b $P < 0.05$ for captopril/hydralazine treated vs untreated group; c $P < 0.05$ for hydralazine-aortic constriction-treated vs sham hydralazine-treated group. (Reproduced from Reddy *et al.*, *Mol. Cell. Biochem.*, 1996, **155**, 1–11.)

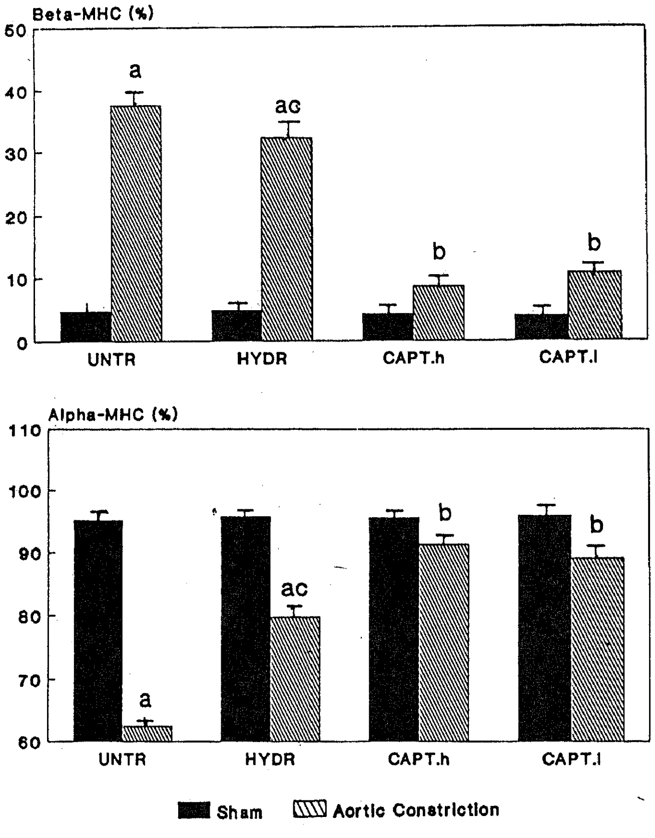

Figure 7. Effects of chronic treatment with captopril or hydralazine on the distribution of myosin heavy chain isogenes in the ventricular tissues in response to cardiac pressure overload induced by abdominal aortic constriction. Values are means ± S.E.m. $n = 5$–8 per group. Sham (filled bars); aortic constriction (hatched bars); UNTR, untreated; HYDR), hydralazine (15 mg/kg, p.o.); CAPT.h, captopril-high dose (100 mg/kg, p.o.); CAPT.l, captopril-low dose (1.5 mg/kg, p.o.), a $P < 0.05$ vs sham; b $P < 0.05$ for captopril/hydralazine treated vs untreated group; c $P < 0.05$ for hydralazine-aortic constriction-treated vs sham hydralazine-treated group. (Reproduced from Reddy *et al.*, *Mol. Cell. Biochem.*, 1996, **155**, 1–11.)

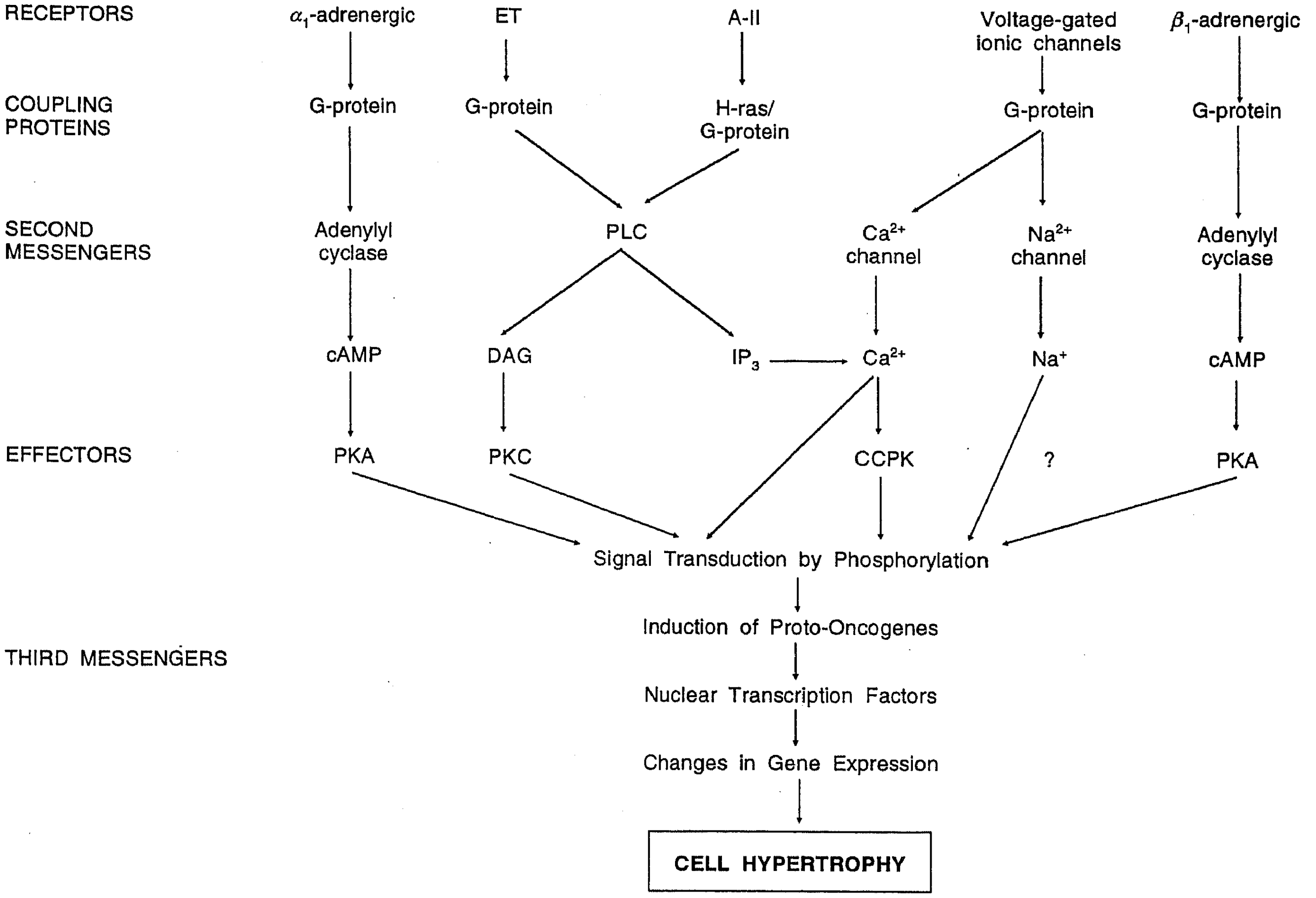

Figure 8. Diagrammatic representation of signal transduction pathways of stretch (ionic channels) α_1-, β_1-adrenergic and angiotensin-II (A-II) receptor-induced cardiomyocyte hypertrophy. (Reproduced from Reddy *et al.*, *Indian J. Pharmacol.*, 1996, **28**, 58–71.)

transforming and fibroblast growth factors (TGF and FGF) have been implicated in signalling processes in cardiac myocyte[287,288]. TGF-β induces re-expression of genes of the 'fetal' isoforms of MHC and actin[277] in normally developing and overloaded heart[289,290]. Growth factors such as α-FGF and TGF-β have been demonstrated to induce cardiomyocyte hypertrophy[291,292]. Insulin like growth factor-1 (IGF-I) induces hypertrophy with enhanced expression of muscle-specific genes in cultured cardiomyocytes[293] and elevated levels of IGF-I in acromegalic patients associates with cardiac hypertrophy[294].

Endothelins

Endothelin-1 (ET-1), a vascular derived peptide factor is another candidate to promote hypertrophy. ET-1 stimulates cardiocyte hypertrophy, sarcomere assembly, expression of proto-oncogenes and ANF. It also simultaneously increases phosphoinositol (PI) hydrolysis and consequent accumulation of IP_3 and DAG[295]. However, the exact role of ET-1 and the effect of mechanical stress on endothelin level are still obscure.

Molecular signalling transduction pathways

Intracellular signalling systems that are involved in cardiomyocyte growth and hypertrophy include oncogenic *H-ras*[296] and G-protein-linked hormone receptors[297] and their membrane enzymes including adenylyl cyclase and phospholipace C (PLC). cAMP induces the hypertrophy in cultured cardiac myocytes and increases the protein synthesis as well as ribosome formation[211,298]. Increase in PKA, PKC, and intracellular Ca^{2+} transduce signals by phosphorylation. These signals induce nuclear proto-oncogenes and transcription factors (Figure 8) which produce permanent changes in gene expression, leading to myocardial cell hypertrophy[228]. Stretch is the main trigger at the origin of the signal transduction, but several hormones such as NE and A-II may also play an important role. Furthermore, interactions between stretch and hormonal stimuli can potentiate each other and result in enhanced signal generation[299].

Recent studies showed that two distinct guanosine triphosphate-binding proteins, oncogenic *H-ras* and *Gq* mediate the hypertrophic response[236,297]. Furthermore, a dominant interfering *H-ras* mutant ablated the α-adrenergic induction of ANF expression[236]. The two G-proteins are part of the signalling pathway that couples the angiotensin and α-adrenergic receptor to the fetal gene programme (Figure 8). The role of the PI as a transducer to hypertrophic signals is fully documented[116,138]. There is evidence that Na^+-current, cAMP and the cytoskeleton may also play a role in signal transduction[113]. However, the precise molecular mechanism of proto-oncogene induction and fetal gene switches of the hypertrophy stimuli are far from clear.

Conclusions and future directions

Cardiac hypertrophy is an adaptive mechanism in response to disease states that produce increased work load. It involves an increase in myocardial cell size, proliferation of supportive tissues and other associated structural changes. Alterations in gene expression play an important role in this adaptive process, including alterations in contractile protein content and composition, the induction of a programme of embryonic gene expression such as ANF, and the expression of proto-oncogenes and other IEGs. The signalling mechanisms that activate and regulate these various alterations in cardiac gene expression during LVH are far from clear. Mechanical stretch directly induces gene expression in cardiomyocytes. Information about the genes that determine cardiac specificity during physiological development is still necessary to understand gene regulation during hypertrophy. Whereas in skeletal muscle both E-box dependent and E-box independent pathways exist, only E-box independent pathways have been found in cardiac muscle so far. Knowledge of cardiogenesis can be applied to target the expression of transgenes to specific cardiovascular compartments, and will provide a molecular framework to understand the development of cardiac malformations. The unraveling of the sequence of molecular events that initiate hypertrophic growth might provide the basis for the understanding of the development of decompensated heart failure. Identification of appropriate molecular markers will result in an important breakthrough in unfolding pathophysiology of LVH.

Although α-adrenergic stimulation is a potent stimulus for growth in neonatal ventricular cells, its role in hypertrophy in intact adult myocardium is not understood. The RAS appears to be an important contributing factor to the development of LVH. Our studies with ACE inhibitors, and others have shown a clear tendency in reversing the cardiac structural changes. Angiotensin-II has been suggested to work as an autocrine/paracrine factor regulating growth of heart and LVH by augmenting the cardiac gene expression. However, we still do not know whether it is the local or systemic A-II that is important in cardiac growth. Recently, we have shown that cardiac RAS plays a critical role in the pathogenesis of LVH. Nonetheless, A-II causes hypertrophy of cardiac myocytes and mitogenesis of nonmyocytes via AT_1 receptor stimulation. Elucidation of the mechanisms of A-II-induced cardiac growth and hypertrophy deserves further investigation because A-II is clearly emerging as one of the most critical mediators of cardiac hypertrophy *in vivo*. Future studies may permit us to design novel therapeutic strategies to heart failure.

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Ecology of desert mammals

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The Thar desert of western Rajasthan exhibits a rich mammalian diversity. In recent years, however, the number of large mammals, artiodactyles and carnivores, has drastically dwindled due to expansion of human population. They are being replaced by small mammals, shrews and rodents. The highly adapted *Gerbillus gleadowi* is a predominant species in the extreme desert largely dominated by sand dunes. The sandy plain in the central desert is the most species-rich habitat and is inhabited by 68 mammal species. Certain rodents like *Cremnomys cutchicus* and *Mus phillipsi* are found in the rocky habitats. The transformation of desert grasslands to irrigated agricultural fields is changing the biodiversity scenario in the desert. Consequently, typical desert-adapted, endemic mammals are vanishing and they are being replaced by mesic elements. Behavioural adaptation of mammals for survival in the inhospitable desert environment is discussed in this paper.

THE Indian Thar desert is located on the south-eastern fringes of the Great Palaearctic Desert, extending over two continents, from Sahara to Gobi desert. Sixty per cent of the Indian Thar is situated in western Rajasthan. The Archaean Aravalli range borders it in the east. The time and origin of the Thar desert still remains a disputed subject. On the basis of archaeological and historical evidences it was regarded to be of recent origin, xeric conditions having been established due to unlimited exploitation and destruction of vegetation[1,2]. However, recent geological[3], geomorphological[4] and palynological[5,6] studies point out that Thar is a very old desert and its aridity has been established over a long and continued period. The floristic and faunal evaluation of the desert biota confirms the latter view[7,8]. The arid region of western Rajasthan is not a vast stretch of sand dunes, but is interspersed with low hills and gravel plains. Human habitations are scattered all over since it is the most populated desert of the world, 78 humans per km^2. Consequently, the desert presents an interesting intermingling of habitats occupied by a species-rich mammalian fauna.

Mammalian abundance

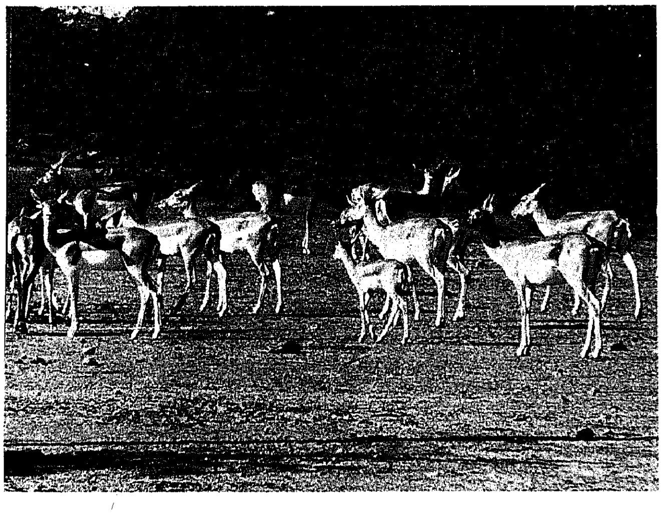
During the beginning of the present century, when human numbers were low, the desert region sustained a great preponderance of larger mammals. The now-extinct cheetah, *Acinonyx jubatus* was distributed throughout the desert and was commonly found around Jaipur[9]. Black buck, gazelle and wild boar have also been widely reported[10]. Herds of two hundred wild ass, *Equus hemionus khur* were reported from Bikaner and Jaisalmer[11]. The medium-sized mammals, the caracal, wolf, jackal, desert fox, desert cat, plentiful up to 1950, have almost vanished. This is mainly due to shrinkage of their native habitat, which is now being taken over by man for agriculture. The larger mammals are also being replaced by smaller mammals like insectivores and rodents. However, the blue bull, *Boselaphus tragocamelus* has benefited by the expansion of agriculture. This is because they are not killed on sentimental grounds. During the 1950s and the 1960s, the house shrew, *Suncus murinus* was collected only from residential premises and their vicinity. Later, during 1970–1971 trapping of small mammals was taken up throughout the desert of Rajasthan. It yielded a few shrews from crop fields, but our recent studies (1993–1994) revealed a high preponderance of this insectivore, especially in the crop fields[12]. The house shrew has apparently altered its ecological niche from houses to crop fields. Likewise, with the fast-changing landuse pattern, the density of several small mammals has enhanced like that of the bush rat, *Golunda ellioti* and the metad, *Millardia meltada*, which were seldom collected earlier. Moreover, a few species which did not occur in the desert have invaded it from Peninsular region, like the bandicoot, *Bandicota bengalensis* and the little mouse, *Mus terricolor*[13] which inhabit the irrigated crop fields. The mammalian scenario in the xeric environment is fast changing with man's intervention.

Habitat preference

The sand dune-dominated, tree-less, 100 mm rainfall region in the extreme western zone of Jaisalmer and Barmer districts is largely inhabited by the hairy-footed gerbil, *Gerbillus gleadowi*, which is endemic to the Thar desert. Its preponderance in the region is astonishing, about 40 gerbils/100 snap traps/24 h compared to 1–8 rodents per 100 traps per 24 h in other parts of the desert. The nocturnal species has been found to be one of the most adapted rodents as it is highly salt-tolerant and is more renal efficient than any other desert mammal[14].

Another finding we have made is that the porcupine occurs among tall sand dune systems of the extreme desert[15]. Elsewhere, *Hystrix indica* inhabits rocky habitat. Is this isolated population thriving in the deep desert, probably since times prior to deposition of sand and when it was a continuous stretch of hills? Similarly the relict Deccanean rock rat, *Cremnomys cutchicus*, occurring on every hill scattered throughout the Thar desert of Rajasthan, is at present the most abundant small mammal over the Aravalli range[12]. The central desert receiving average annual precipitation up to 400 mm is relatively a species-rich region because of diversity of habitats, the rocky outcrops, gravel plains, lakes, beds of ephemeral rivers and village complexes. In the rocky habitat, thickets of *Euphorbia caducifolia* provide shelter to the desert hare *Lepus nigricollis* and several species of mice. Crevices in the rocks are the special niche of the most abundant *Cremnomys cutchicus*. The larger caverns and caves are occupied by about 16 species of microchiropteran bats, only sometimes by rare species like wolf, jackal, hyaena and panther. Over the hillocks, which are protected from grazing livestock, porcupine is also found.

Village settlements occur in every habitat in western Rajasthan and they provide an important habitat for mammals. Whereas the magnificient black buck has almost vanished from the desert region, its free-living populations are still found around villages of Bishnoi people, who regard it sacred and protect it. Along with black bucks, the Indian gazelle, *Gazella bennetti* also congregates around Bishnoi habitations. The desert cat, *Felis silvestris* has so much intermingled with the domestic cats that it is difficult to find a pure-bred wild form. Old buildings, forts and deep wells are inhabited by microchiropteran bats. The two megachiroptera, the flying fox, *Pteropus giganteus* and the smaller frugivore *Cynopterus sphinx* are found over large trees in gardens. The striped squirrel, *Funambulus pennanti* is the most common mammal in the vicinity of human dwellings.

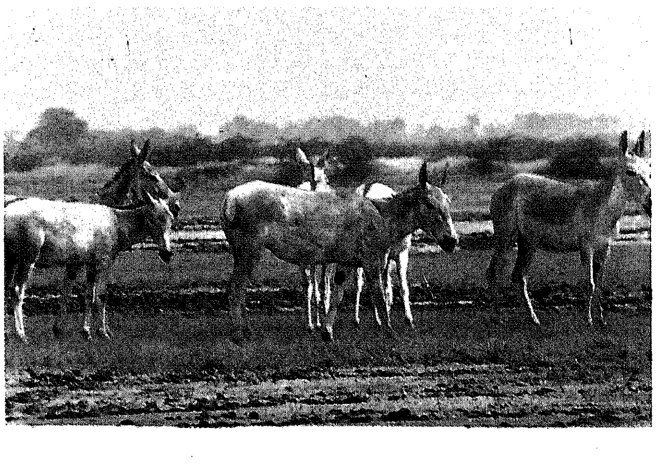


Early during the century the black bucks (*Antelope cervicapra*) were found in the desert region in groups of 5000 to 8000. At present, however, free-roaming antelopes are found only in the vicinity of Bishnoi village complex. This community of desert dwellers protects wildlife and trees on religious grounds. It is an unique example of human–animal relationship. (Photo: B. S. Lamba).

The northern desert, Sri Ganganagar and Hanumangarh districts have been transformed from open desert grasslands to irrigated cropping with the advent of Gang Canal during the 1930s. At present the Himalayan water is flowing into the desert up to Jaisalmer through the Indira Gandhi Canal. Extensive agriculture has already come up very close to Bikaner. In this irrigated region the moisture regime pattern in the sand has been totally altered, it is moist all the year round. Consequently, the vegetation type has changed and typical deserticolous animals are being replaced by mesic forms. The gazelles have migrated to dry zone and they are being replaced by the blue bull. The highly-adapted desert rodents (*Gerbillus*, *Meriones*) have been replaced by mesic *Bandicota*, *Mus* and *Millardia* (Table 1). The latter species are serious pest of standing crops and very dangerous from the epidemiological point of view as they are very susceptible to the plague bacillus. Moreover, the desert is losing its endemic xeric fauna, which is not found anywhere else in India, due to altering landuse pattern.

Desert adaptations

The desert animals and plants are confronted with thermal extremes, paucity of drinking water and food, and scanty shelter. The desert mammals have evolved a series of adaptive strategies for their survival in the inhospitable desert environment. About 80% mammals inhabiting the arid zone are nocturnal, and by restricting their activities to cooler nights, they avoid the heat of the day. Moreover, when the air temperature falls below −5°C, most of

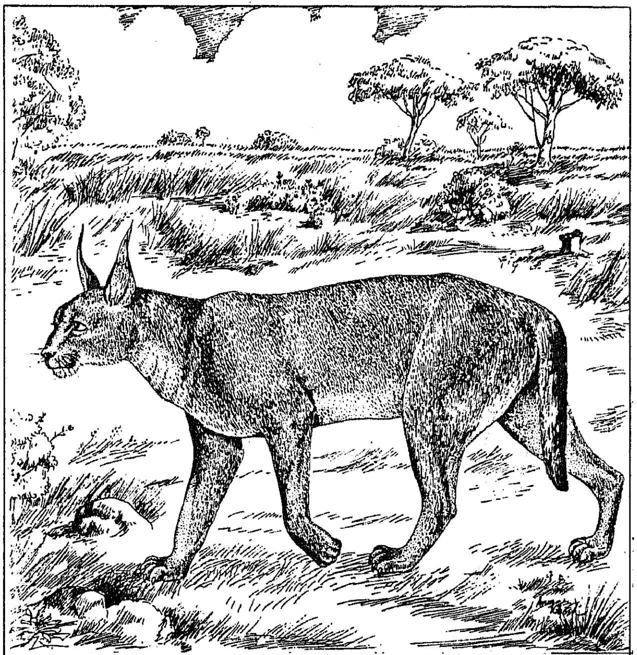


Herds of wild ass occurred in Bikaner and Jaisalmer before the turn of the last century. At present, *Equus hemionus* is restricted in the Little Rann of Cutch. (Photo: S. P. Sanyal).

them cease their activities and enter their hideouts. It has been observed that during summer, the Indian gerbil, *Tatera indica* ventures out of its burrow at about 17.30 h and the peak in its activity is reached at 23.00–24.00 h and terminates early in the morning. However, during the winter season when the nights are very cool, the peak activity is advanced, soon after dusk, at about 19.00 h and ceases by 24.00 h. By restricting its outside-burrow activity, it avoids exposure to the mid-night extreme cold. Likewise, the diurnal desert gerbil, *Meriones hurrianae* also shifts its diel activity into Bimodal pattern during summer months: during mornings and evenings. However, in winter it forages all through the day. Desert mammals shift their diel activity to comfortable periods to avoid the extremes of temperature.

Table 1. Succession of species replacement in the desert region under Indira Gandhi Canal Command

Period	Mammals (arranged in the order of abundance)
Prior to incoming or irrigation	*Gazella, Gerbillus, Meriones, Tatera*
25 years after incoming of irrigation	*Gazella, Tatera, Millardia, Meriones*
65 years after	*Boselaphus, Millardia, Tatera, Mus, Bandicota*



The caracal (*Felis caracal*). Locally known as *Siyagosh*, the caracal was a rare animal even at the turn of the last century. Distributed in deserts of Iran, Arabia, Pakistan and the Thar desert, at present, it is considered as a threatened species. Caracal is stout mammal with long limbs, larger than a cat, long ears with a tuft of black hair on the tip. Inhabits open grasslands and feeds on birds and small mammals. It has been reported to spring upon low flying birds up to about two metres. It is so powerful that it can chase and kill a gazelle. People used to tame and train it to catch peafowls, cranes, hares and other medium-sized mammals. The caracal is extremely agile and swift. When let loose amongst pigeons feeding on the ground, the caracal strikes down 10–12 birds before they can escape by flight. Alas! this magnificient animal is on the verge of extinction in the Indian Thar desert [Diagram: Arun Ghosh].

The smaller mammals (56%) have adapted to live a fossorial life; insectivores (4 species), medium-sized carnivores (13), pholidote (1) and rodents (17). The temperature inside the burrows does not fluctuate as much as the air temperature does during the day (Figure 1). When the desert gerbil, *Meriones hurrianae* develops hyperthermia due to continuous exposure to sun, it intermittently visits the cooler environments of the burrow and unloads the excessive heat. This may be the reason that diurnal rodents excavate extensive, multi-tier burrows as compared to the nocturnal ones. All the mirochiropteran bats (25% of mammal species) inhabit cooler environments of caves, tunnels, and ruins of buildings.

Almost all the mammals are very versatile as far as their food habits are concerned. It is not surprising to find that 90% of the stomach contents of the insectivorous *Suncus murinus*, examined all through the year, constituted leaves of an inedible shrub, *Mimosa hamata*. Likewise, stomach contents of carnivorous desert fox, desert cat and jackal were found full of fruits of cucurbits

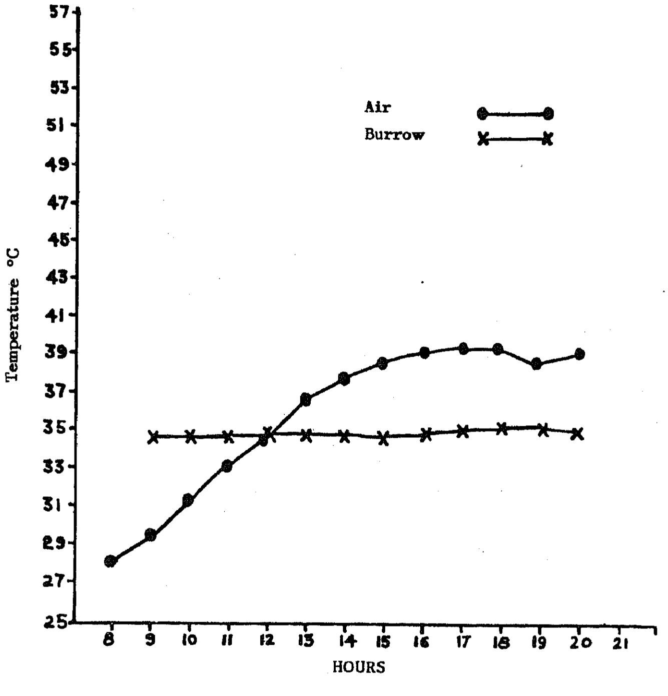


Figure 1. Diurnal variations in air temperature and inside burrow of desert gerbil.

Table 2. Distribution of litter size of the desert hare during a year. The litters of 3 and 4 young occurred only during monsoon months

	No. of litters of different sizes			
Month	1	2	3	4
January	5	–	–	–
February	1	–	–	–
March	3	–	–	–
April	3	–	–	–
May	4	5	–	–
June	0	0	0	0
July	–	5	2	1
August	–	1	3	2
September	2	1	3	1
October	–	5	2	1
November	9	1	–	–
December	1	–	–	–

Table 3. Zoogeographical affinities of mammal in the Thar desert

	Number of species and their affinities		
Order	Saharan	Tharian	Oriental
Insectivora	2	–	2
Chiroptera	8	1	9
Primates	–	–	2
Pholidota	–	–	1
Carnivora	11	–	5
Perissodactyla	–	1	–
Artiodactyla	2	–	3
Lagomorpha	1	–	–
Rodentia	8	1	11
Total	32	3	33

Macroendemism: 4.4%
Microendemism: 20 subspecies, Tharian.

and berries of *Ziziphus nummularia*. The seedivorous-desert rodents switch over to insects during the summer months when vegetation is without any water content. Insect bodies contain about 80% water. The shift to insect food during summer also helps them in osmoregulation. The gerbils show spectacular fluctuations in feeding on various plant parts over the year depending on their availability in the desert ecosystem. Our study[16] revealed that during winter, when the seeds are available after flowering season, the gerbils feed on maximum quantity of seeds, 60% of the total. During monsoon, when green food is available, blades of grasses and leaves of herbs are consumed (30–40%). But during summer, they totally depend on stem, rhizomes of sedges and grasses; and insects. Moreover, during summer when food is scarce, the merion gerbils have been observed to scent-mark dry clumps of grasses, probably to indicate their territoriality over it to other conspecifics. Omnivory is one of the major strategies for survival in the desert.

Most mammal species coincide their maximum breeding time to monsoon months because of the prevalence of conducive environmental condition and availability of green food. Our field data has clearly shown that the survival rate of the young born during the latter half of the year is significantly ($P < 0.01$) superior to those born during the first six months of the year. Moreover, among the mammals which litter all the year like the desert hare, *Lepus nigricollis dayanus* (Table 2), the largest litters are born during July to October, the rainy months.

Zoogeography

A detailed analysis of mammalian distribution in African and Asian deserts has revealed that some genera like *Meriones* and *Gazella* are spread from Sahara to Gobi desert and find their south-eastern limit in the Thar desert. A few species are Turano-Tharian and about 50% species exhibit oriental affinities (Table 3). Three mammalian species and 20 subspecies are Tharian in origin.

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Desert climate and its dynamics

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Basic characteristics of desert climate are discussed in relation to aridity indices based on water balance and energy balance consideration. The dynamical aspects of the underlying causes in terms of tropical Hadley cell, orographically forced subsidence and large scale ocean–atmosphere interactions are elaborated. Climatological aspects of the Thar desert (Rajasthan) in India and a review of the present and past climates of the region are presented. Interannual fluctuations of arid conditions are discussed in terms of several parameters. Local and regional dynamical aspects of the desert climate as revealed by recent field experiments are discussed. Anthropogenic causes of desertification are discussed in terms of the biogeophysical feedback processes such as albedo, soil moisture and roughness length. Recent work of several workers, employing general circulation modelling experiments to study the sensitivity of land surface processes to desert climate are briefly reviewed.

DESERTS or arid zones are one of the important components of the Earth's environment. They are fragile ecosystem where vegetation is absent or scarce as moisture is insufficient to support it. They are progeny of global climate system under the influence of large scale land–ocean–atmosphere–cryosphere interactions.

Two systems of climatic classification are usually followed after Koppen and Thornthwaite. In the Koppen's[1] system desert or arid climate is characterized by regions where mean annual precipitation is low (< 250 mm) and surface air temperature is high. The Thornthwaite's[2] system is based on water balance or hydrological approach and compares the mean annual rainfall against mean annual potential evapotranspiration (PET). Deserts (arid and semi-arid regions) occupy nearly 30% of the land area of the Earth and nearly one sixth of world's human population live in these environments. Obviously extensive scientific work on various aspects of the desert environment has been done. Our objective is to consolidate briefly information on desert climate, mostly restricted to near-surface climate.

The following indices are usually used to define aridity or moisture availability:

$$\text{Thornthwaite's Index } I = \frac{\text{Mean annual precipitation } (R)}{\text{Mean annual potential evapotranspiration}}.$$

Different classes of arid zones are described as – (i) Hyperarid: $I < 0.05$ (almost absence of vegetation); (ii) Arid: $0.05 > I < 0.20$ (dry zone where perennial agriculture is not possible); (iii) Semi-arid: $0.20 < I < 0.50$ (shrub lands where rainfed agriculture is practiced).

Moisture index (I_m) is usually defined as:

$$I_m = \frac{100\,S - 60\,d}{\text{PET}},$$

where S is water surplus and d is water deficiency. A region is considered having desert climate if I_m is below – 60 and this limit on I_m generally agrees with Koppen's classification BW for the deserts.

An entirely different method of quantification of aridity is after Budyko[3] and follows the energy balance approach in which net radiation (Q^*) received at the surface of the earth on annual basis is compared against the mean annual precipitation (R). (Q^*) is given by the well-known energy balance equation

$$Q^* = (Q + q)(1 - x) - (\text{LWu} - \text{LWd}) = H + LE + G,$$

in which Q and q are the direct and diffuse solar radiation received at earths surface, x is the albedo of the surface, LWu and LWd are the upward and downward long wave emissions by the earth–atmosphere system respectively. The net radiation is used in three major ways, viz. (i) in transporting sensible heat (H) upward by turbulent eddies, (ii) in removing water/moisture from surface by evaporation and transpiration (LE) and, (iii) in conducting heat into the soil (G). The small portion of energy used in photosynthesis is neglected for the energy balance purpose. Lettau[4] defines a dryness ratio by comparing Q against heat required to evaporate rainwater

$$\text{Dryness ratio} = \frac{Q^*}{LR},$$

where L is latent heat of vaporization.

For hyperarid regions dryness ratio is > 10, for arid regions it is 7 to 10 and for semi-arid regions it is 2 to 6.

Global distribution and mechanisms of deserts

In Koppen's classification the desert climate is designated as BW and these regions are surrounded by the climate type BS. The marine deserts, where rainfall is also scanty, are designated as BM. The world's major deserts in different continents are: (i) Sahara, Saudi Arabia, East Africa, Namib, Kalahari in African continent, (ii) the Thar, Taklamakan and Gobi in the Asian continent, (iii) the Great Sandy and Simpson deserts in Australia, (iv) Mojavet, Sonaran and Chiliuahan in North America, and (v) Peruvian, Atacama, Patagontan in South America. Oceanic deserts exist in the equational central and eastern Pacific where the SST is < 26°C and under the sub-tropical high pressure belts in north and south Atlantic and Pacific Oceans, in the South Indian Ocean and over Western and Central Arabian Sea. Coastal marine deserts exist off the major ocean upwelling regions or where cold ocean current prevail. Any geographical atlas depicts the distribution of major deserts over land. From this distribution it becomes apparent that most of the deserts over the Earth are located within the descending limb of the direct Hadley circulation whose ascending limb on annual average basis lies in the near equational region. The ascending and descending limbs shift poleward (equatorward) during summer (winter). Thus in the sub-tropical belts some rains occur in the desert regions during summer due to moisture incursion with the poleward excursion of the Intertropical Convergence Zone (ITCZ) or Monsoon Trough. A few rain spells may also occur in winter under the equatorward extension of baroclinic disturbances. The subsidence motion under the sub-tropical high pressure belts does not favour convective motion or cloud formation. The sub-tropical deserts are further desiccated if a region lies under the influence of cold currents or off oceanic upwelling regions such as the Namib desert in South Africa, the Atacama and Peruvian deserts in South America and the East African desert of Somalia. The inner continental deserts within or close to the sub-tropical belts are also sustained by the downward motion on the leeward side of major mountain barriers such as the Taklamakan desert in Asia and Patagontan desert in South America. The aridity of the Interior Karnataka, parts of Maharashtra and Andhra Pradesh States of India is also ascribed to the rain shadow effect of the Western Ghats.

Characteristics of desert climate

Low rainfall and high temperature are the most conspicuous characteristics of desert climate. The mean annual aridity indices are different for different land deserts of the world and the aridity is highest for the core Saharan and Peruvian deserts, followed by the Saudi Arabian, East African and Gobi, Australian and South African deserts. It is low for India's Thar desert and the North American deserts of California and Mexico. It is observed (Berkofsky[5]) that there are regions in the Saharan, the Australian and the Namibian deserts where the annual rainfall is almost negligible < 50 mm. Compared to that most of the Thar desert in India receives considerable rainfall (100–300 mm).

Present and past climates of Thar desert of India

The Thar desert of Sind Province of Pakistan and Western Rajasthan region of India is a meteorologically homogeneous region where physiographic and anthropogenic conditions are somewhat comparable to the contiguous Saharan region to its West. The Aravallis range in Rajasthan defines the eastern limit of the Thar. Most spectacular amongst the land forms in West Rajasthan are the sand dunes which contribute about 40% of area of the region. The dunes are not contiguous and dune-free corridors exist. Some of the dunes are stabilized whereas others are less stable and wind erosion is a common feature from dunes to dune-free areas. The Thar region is under the influence of the Indian South-west monsoon (or summer monsoon) during July and August and occasionally rain-producing weather disturbances like 'depressions' and 'lows' penetrate up to parts of Rajasthan triggering rain events in the Thar. The precious rain events in Thar desert support a large population with above average growth rate than the average growth rate of India.

Climatology over the Western Rajasthan

The climate of the Western Rajasthan is controlled during the summer monsoon (June–September) by the seasonal heat low over Sind–Western Rajasthan region and during winter (December–March) by the Siberian anticyclone at the surface level. Thus the wind flow near the surface is from South West/West during the summer monsoon and from a general NW direction during the winter. At the upper tropospheric level wind flows from the east during the summer monsoon and from the west in the winter season. In the transition months of October and November in autumn, the pressure gradient is very slack. In the spring/early summer during April and May intense heating during the day, coupled with existence of pressure gradient in the formative stage of the summer time heat low, supports strong dust-raising winds. The almost clear weather pattern during late January to June is interrupted by the passage of a western disturbance which occasionally affects the weather of Western Rajasthan such that a few mild rain events occur during January–March and dust storms from April to June.

Rainfall

The main rainy season is during July and August when almost 80% of the annual rain occurs. Figure 1 shows the annual distribution of rains for West Rajasthan along with the coefficient of variation. The monsoon seasonal rainfall follows the annual rainfall and decreases from about 500 mm over East Rajasthan to about 100 mm over extreme western portion of West Rajasthan. The rainy days also decrease from East to West Rajasthan. The coefficient of variation is also higher over West Rajasthan. The rainfall is mostly convective in nature such that an intense weather system even in one day produces 50% or higher of the annual rainfall in one day. The highest one-day rainstorm of Rajasthan has occurred over Banswara in East Rajasthan (559 mm). Figure 2 shows the one-day probable mean precipitation (PMP) for Rajasthan. Dhar *et al.*[6] have studied the depth–area–duration of some of the severe most rainstorms that have occurred over Rajasthan for 1- to 5-day and have reported that for 100 km^2 area the 1-day rain depth has been approached in the rainstorm on 19 July 1981. The same rainstorm produced a rainfall of 265 mm at a station in Nagaur district in West Rajasthan. The mean annual rainfall of West/East Rajasthan is given in Table 1.

Length of the monsoon rainy season

The monsoon rains begin in West Rajasthan by 10–15 July and end by 7 September. In the mean, the season lasts for about 50–60 days. Inter-annual variations in the duration of the season are marked due to early arrival and late cessation of the rains and in extreme cases the season can last between 40 and 70 days. This large fluctuation has marked effect on the quantum of rainfall in this desert.

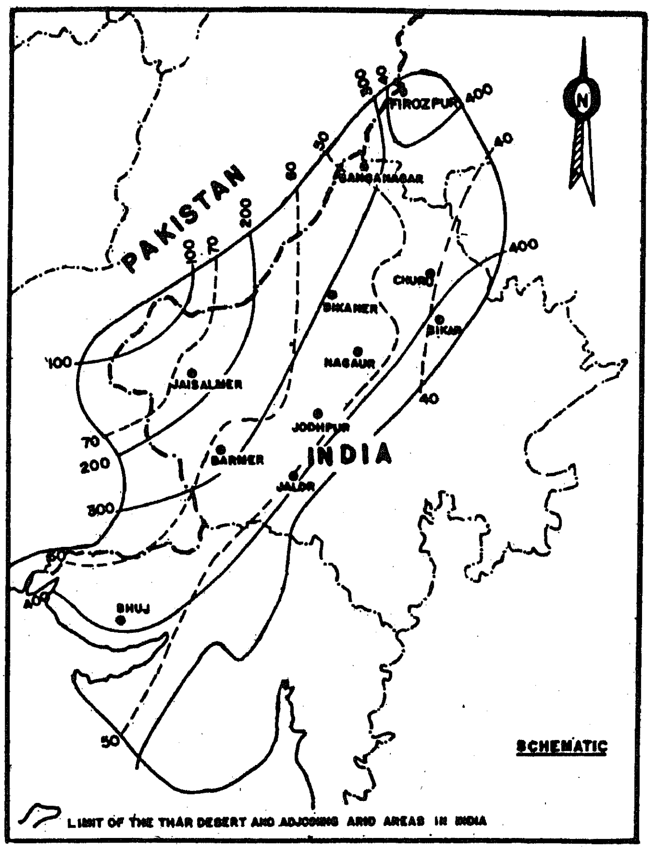

Figure 1. Mean annual rainfall (in mm, full lines) and its coefficient of variation (in %, dotted line) over West Rajasthan.

Fluctuation in aridity index

Nityanand Singh *et al.*[7] have examined the year-to-year fluctuations of the area under less than 560 mm of annual rains, which they call as arid area, over India, North India and Peninsular India. For North India (north of 20°N), the arid area, as defined above, covers 43×10^4 km^2 with a standard deviation of about 14.8×10^4 km^2 (coefficient of variation, 34.3%). This area shows expansion and contraction on inter-annual basis (Figure 3) and in an extremely dry year like 1918 or 1987 major part of north India experienced arid conditions whereas the area contracted to cover only parts of West Rajasthan in extremely good monsoon year like 1917 or 1961. In recent years there is a declining tendency in the arid area with a westward shift in heavy rainfall events. Figure 4 after the work of Ramakrishna[8], shows that Thornthwaite's mean moisture index line of – 80% has shown east-west fluctuations around its long term normal position – the protrusion to west being somewhat large in the decade of 1971–80 (wet decade). This and the rainfall analysis of Rajasthan suggest that in recent decades the Thar desert appears to have shrunk instead of expanding to the east. Mean annual aridity index calculated by Parthasarathy and Rakhecha[10] is given in Table 2 along with annual rainfall and annual maximum and minimum temperatures.

Droughts in Rajasthan

Table 3 shows the individual drought years (annual rainfall departure from long term normal < 25%) over the meteorological sub-divisions of West and East Rajasthan as well as the drought years which are common to both the sub-divisions.

It is clear from Table 3 that West Rajasthan is more prone to drought and several of the drought years are not common between the two sub-divisions even though they are contiguous zones. Besides, the common drought years are mostly those in which the country as a whole had suffered from bad monsoon (rainfall < one standard deviation for the country as a whole). These drought years are those in which India is affected by the adverse effects of planetary scale ocean–atmosphere coupled system ENSO phenomena. Sikka[11] was among the first to suggest ENSO–monsoon relationship and since then many researchers have further investigated this relationship. Rakhecha[12] has listed the years of excess rainfall (> 50% normal) and deficit rainfall (< 50% normal) years for the period 1871–1990.

Stability and moisture content

The Thar desert of India witnesses extreme surface air temperature during the year. The maximum temperature in May–June touches 50°C and the minimum temperature in January–February reaches $-3°C$ or even less in extreme cold wave conditions. The lowest temperature recorded at Jaisalmer is $-5.9°C$ with the mean monthly extreme lowest temperature of $-2°C$ in January. Whereas the diurnal temperature range in summer and winter is normally about 20°C, it reaches 25°C under extreme conditions.

The soil temperature at surface in summer is even 10°C warmer than the maximum surface air temperature near noon and it is about 3°C colder than the minimum air temperature in winter. This indicates that super adiabatic lapse rate (highly unstable lapse rate) prevails during the day time in summer and a strong inversion (highly stable lapse rate) exists in winter near the surface from dusk to early morning. Ramakrishna *et al.*[13] conducted a special experiment from May 1987 to April 1988 at stable and unstable sand dunes in the area to measure soil temperature and surface air temperature. The data showed that the maximum soil temperature of 55°C or more was recorded in May 1987 on all faces and again temperature even over 60°C, were recorded in September 1987. During November there was a considerable fall in the maximum temperature for the soil and air. Thus, maximum difference in soil to surface air maximum temperature recorded during the experiment reached a value of 21.2°C at the crest during June 1987, showing high super adiabatic lapse rate over the dune surface during day time. Minimum soil temperature during early morning was highest in May 1987 (about 28°C) and it showed considerable fall in November 1987. The minimum air temperature was higher than the minimum soil temperature, indicating inversion lapse rate (stable) from the soil to the surface air layer during night/early morning. The lapse rate above the surface layer is nearly adiabatic up to about 600 hPa in May and June during day hours under the influence of intense solar heating coupled with thermal and mechanical turbulence. During the monsoon months of July–August the lapse rate is mostly between dry-adiabatic and moist adiabatic, favouring conditional instability, which promotes moist convection under favourable conditions. Rajkumar *et al.*[14] while analysing MONTBLEX data at Jodhpur have found that even during the monsoon season, when the ground is wet and the sky is cloudy, the lapse rate in the lowest part of the atmosphere in the PBL is mostly stable during day and night hours. Usually most of the moisture resides in the mixed layer or planetary boundary layer during the monsoon period and it decreases sharply above the low level inversion layer which acts as a lid for the moist processes and suppression of convective clouds. During winter the lapse rate in the lower troposphere is statistically stable under normal conditions except during rain episodes under the influence of western disturbances.

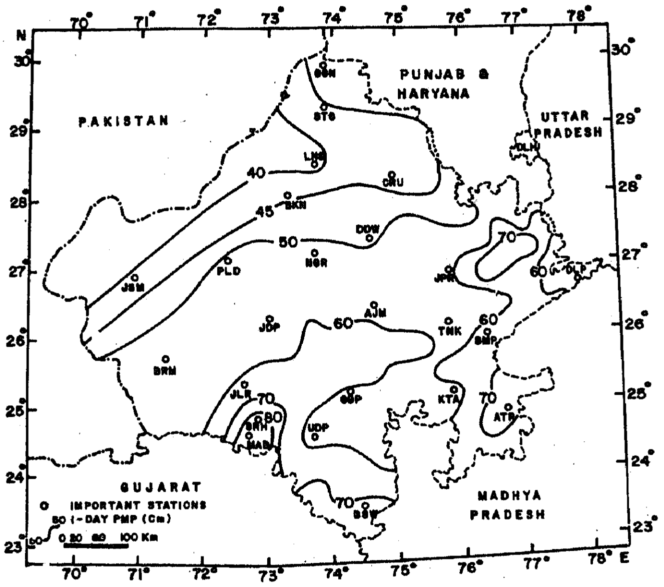

Figure 2. One day probable maximum precipitation over Rajasthan (after Rakhecha[12]).

Solar radiation and sunshine

Thar desert region receives intense solar heating during April to June and again in September and October. The

Table 1. Mean annual and monsoon season rainfall statistics for Rajasthan

Region	Annual rainfall (mm)	C.V. (%)	Monsoon season rainfall in mm (% of annual in parenthesis)	No. of rainy days
West Rajasthan	309	40	275 (88)	17
East Rajasthan	679	26	623 (92)	34
Rajasthan as a whole	468	31	425 (91)	29

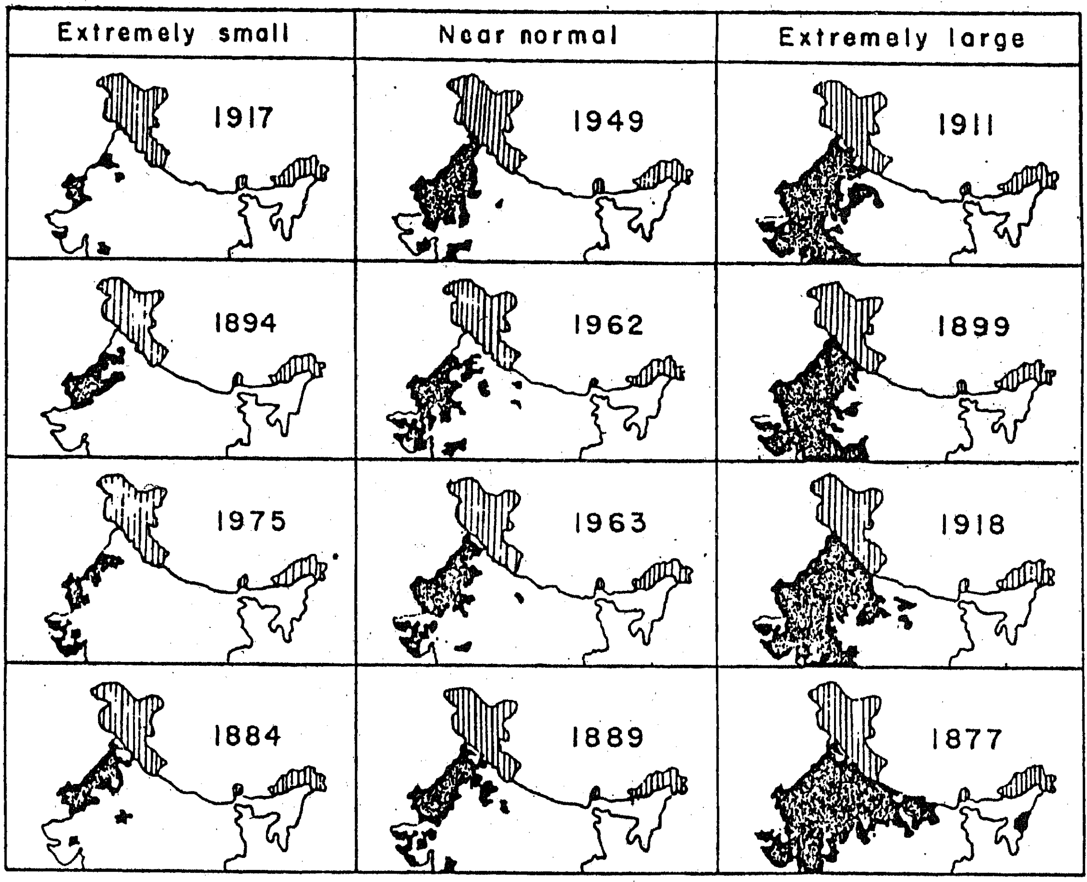

Figure 3. Fluctuations in arid area of North India in some typical years (after Nityanand Singh *et al.*[7]).

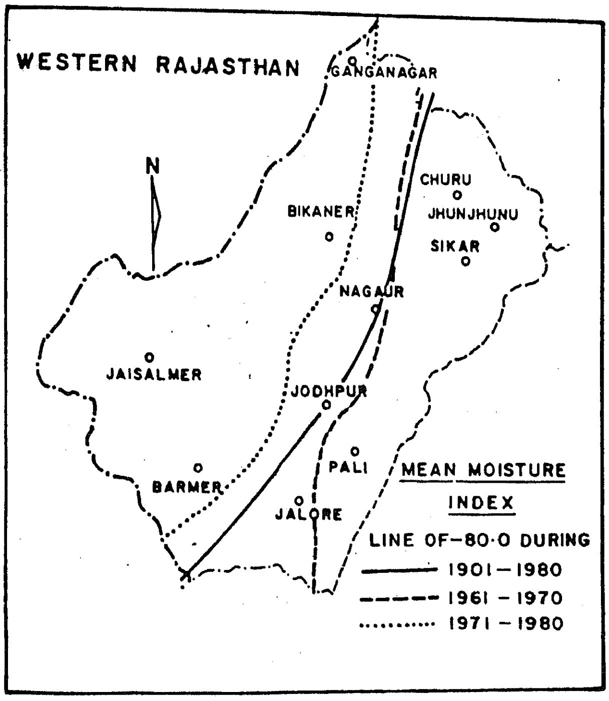

Figure 4. Shifts in the mean moisture index line of – 80.0 over western Rajasthan (after Ramakrishna[8]).

maximum global radiation (direct and diffuse sky) per day is 700–800 cal/cm^2 per day in May and minimum of 400 cal/cm^2 in January with annual average of 560 cal/cm^2 per day. Under overcast sky conditions on a particular day the solar radiation is reduced to 190 cal/cm^2 per day. Solar radiation decreases in July and August and a secondary maximum occurs in October. Dust and clouds are major factors in attenuation of solar radiation. Maximum hours of bright sunshine, reaching 10 h per day occur in May and October[15,16]. According to their estimates[15,16] global radiation of 60 cal/cm^2/h is available for 4 to 5 h per day (1100 to 1600 h), indicating high potential for solar energy in the Thar desert. Desikan *et al.*[17] showed that there is a decreasing trend in solar radiation from 1965 to 1985 over Jodhpur, perhaps due to higher dust load.

Surface winds

Generally the winds near the surface have westerly component during major part of the year. They are stronger over the West Rajasthan than over the East Rajasthan. Surface winds reach a value of 25–30 km/h over West Rajasthan during May–June. Such strong

Table 2. Mean annual aridity index rainfall maximum and minimum temperatures for some stations in West Rajasthan

Station	Aridity index	C.V. (%)	Annual rainfall (mm)	C.V. of rainfall (%)	Annual max. temp. (°C)	Annual min. temp. (°C)
Barmer	-80.5	18	268	58	46.0	6.0
Jodhpur	-75.7	25	361	53	45.0	3.0
Bikaner	-77.5	22	294	55	46.0	2.0
Ganganagar	-77.5	22	214	43	46.0	3.0

Table 3. Drought years for West and East Rajasthan and the common droughts (period 1871–1990)

West Rajasthan drought years	East Rajasthan drought years	Common drought years
1877, 1883, 1885, 1887, 1891, 1901, 1902, 1904, 1905, 1911, 1913, 1915, 1918, 1920, 1925, 1938, 1939, 1941, 1946, 1948, 1951, 1963, 1968, 1969, 1972, 1974, 1979, 1982, 1986, 1987.	1877, 1895, 1898, 1899, 1901, 1905, 1907, 1911, 1913, 1915, 1918, 1925, 1938, 1939, 1941, 1951, 1965, 1966, 1972, 1979, 1982, 1986, 1987.	1877, 1892, 1901, 1905, 1911, 1913, 1915, 1918, 1925, 1938, 1939, 1941, 1951, 1972, 1979, 1982, 1986, 1987.
(30 years)	(23 years)	(18 years)

winds raise tremendous dust from the loose sandy soil of the region and the dust is transported eastward over neighbouring states of India.

Dust layer and dust storms

A dust layer from ground up to 8–9 km height is built up gradually from March to June which affects the horizontal and vertical visibility. Dust depletes solar radiation by 30%. During intense episode of dust-raising winds or following the dust storm day the sun is obscured from view even during the mid-day. The dust (sand) raising capacity of the surface air is proportional to the cube of the wind speed and hence the capacity of the winds to raise sand from the desert increases sharply after a wind speed of 10 km/h. The average soil loss in the Thar desert varies between 30 and 60 kg/m^2 per day during April to June[18].

Dust storms also occur more frequently in West Rajasthan than over the East Rajasthan during April to July, the frequency, however, being highest over Ganganagar (17 days), followed by Bikaner (15 days), Jodhpur (6 days) and Jaisalmer (2–3 days). Dust storms are often accompanied by thunderstorms (dry and wet). The annual number of thunderstorm days is about 10 over West Rajasthan and increases to about 40 over East Rajasthan. Singh *et al.*[19] have studied the hygro-thermal regime during a duststorm period in May 1990 under the MONTBLEX Programme.

Parameters of the hydrologic cycle

The annual PET over West Rajasthan varies from 2000 to 2500 mm and reaches a maximum of over 3000 mm in May–June. PET for monsoon months is about 600–800 mm. As the annual and monsoon seasonal rainfall figures are below the PET, moisture deficit exists over West Rajasthan throughout the year under normal conditions. However for the East Rajasthan moisture surplus does occur during July and August, particularly in years of excess rainfall. Since water at the surface is available only during rain spells in monsoon season and in winter, the evapotranspiration is high only during periods of rain spells. Soon thereafter the water gets evaporated due to high winds and high temperature. In the long dry period, the evapotranspiration is low as the vegetation closes its stomata to restrict transpiration in order to survive itself against moisture stress. Thus the average annual evapotranspiration over the Indian part of the Thar desert is about 400 mm. Since the soil in the desert is sandy, most of the rainfall percolates below the soil and is stored as soil moisture and very little is left for runoff. Even the run off percolates below the soil and the streams dry off down the course rather fast. Studies on soil moisture by Abichandani *et al.*[20] and Gupta[21] show that soil reaches field capacity in intense convective episodes and is charged with water up to even 100 cm depth. While natural hardy vegetation near the surface soil dries up in the dry season, deeper parts of its roots are sustained by moisture supply from the deep soil and with the first rains of the monsoon the desert springs up into life. Ramakrishna *et al.*[22] have discussed in detail about the annual moisture regime variability and its impact on agriculture in Rajasthan desert.

Climatic fluctuations over Rajasthan during instrumental period

Several workers have examined the question of climatic fluctuations over Rajasthan since the first major drought occurred in 1951 after India's independence[23–30]. All the studies suggest that there has been no major shift in the rainfall or temperature climate of Rajasthan. Pant and Hingane[29] showed that there is very little trend in Rajasthan rainfall over the last 100 years. However a small positive trend exists over East Rajasthan and neighbouring region of West Madhya Pradesh, Haryana and Punjab for the monsoon season rainfall and annual rainfall. Based on their analysis Pant and Hingane have rejected the results of Winstanley[31], suggesting that the rainfall of NW India was declining due to the equatorward shift of polar circulation and consequent equatorward shift of the ITCZ. They also conclude that a gradual decreasing trend in mean annual temperature of NW India is observed. The increasing rainfall and decreasing temperature trends are consistent and are attributed cautiously by the authors, to a progressive increase in the irrigated area. Ramakrishna and Rao[32] have also shown trend toward more rainfall, lower temperature and lower incidence of dust-storms in the command area of Indira Gandhi Canal.

Climate change scenario for Rajasthan due to anthropogenic activity

There is considerable worldwide concern about the possibility of climate change in desert regions due to global warming under the increasing greenhouse effect being caused by anthropogenic activity. Several numerical experiments have been performed world-wide for climate change under double CO_2 scenario using equilibrium climate change and employing stand alone atmospheric GCMs and coupled ocean–atmosphere GCMs. Rajasthan is too small a region to be effectively studied through the results of numerical modelling experiments. The result of the experiments could be valid with some certainty for the global climate system and considerable uncertainty exists when they are applied to regions/subregions. Since Rajasthan rainfall is mostly determined by the south-west monsoon of India, any change in its intensity under the double CO_2 conditions would impinge upon Rajasthan rainfall and water resources. Sikka and Pant[33] showed that for the stand-alone GCMs the monsoon rainfall of northern India may increase by 5–10% and the surface temperature may increase by 1–2°C. However, in the latest results of Lal *et al.*[34] using a coupled atmosphere–ocean model and incorporating sulphate aerosol due to projected enhancement of industrialization, the rainfall showed decrease by about 5% and the temperature also showed decrease by about 1°C. In any case these scenarios are only suggestive and the magnitude of projected change is rather quite small, much smaller in comparison to the interannual or interdecadal variability of the meteorological parameters witnessed in the instrumental period. Thus there is hardly any cause for concern with regard to climate change due to greenhouse global warming over India or over Rajasthan desert. The real problem in desert climate is the inter-annual variability. Most of India is situated in the deep moist tropics, with major ascent centred over the warm north Bay of Bengal waters and adjoining monsoon convective regions over the Eastern Gangetic plain in the west and moist Bangladesh, SE Asia and South China in the East. So long as this moist convective regime is maintained by natural atmospheric dynamics, it would suppress advancement of the arid conditions of Western Rajasthan farther eastward into the Gangetic plain.

Appraisal of palaeoclimate of the Thar desert

The Thar desert region, like many other deserts has witnessed climate changes due to natural causes. Thus, it is expected that in the historical and pre-historical pasts, monsoon fluctuations due to natural causes such as change in earth–sun geometry and glacial–inter-glacial episodes lasting for hundreds of years would have affected the climate of the Thar desert. Besides tectonic disturbances in the Aravallis would have affected the courses of river systems such as the Chambal–Yamuna river system and the legendary Saraswati and Drishdawati rivers which once flowed through the desert. Many attempts have been made to reconstruct the palaeoclimate of the Thar desert by employing a variety of multidisciplinary approaches such as physiography[35], pollen data from the desert salt lakes[36], thermoluminescence chronology of fossil sand dunes[37,38], archaeological records and history of past civilizations which flourished in the Ganga–Yamuna–Indus–Saraswati river beds[39], glacial and inter-glacial chronology[40–44] and geomorphologic studies[45–49]. Pant and Maliekal[50] have given good surveys of the past climate changes from last glacial to the quaternary phase of the global climate. According to these studies the past climate history of Rajasthan can be grouped into the following phases:

Phase I (20000–10000 years BP, last-glacial). This was the period of ice age conditions with cold temperature and extreme aridity in Rajasthan. Several numerical experiments, using atmospheric general circulation models support this inference as the monsoon with ice age boundary condition is simulated to be very weak in the models with its northward limit restricted to about 15°N only.

Phase II (10000 to 5000 years BP). Increase in vegetation and lake water levels suggestive of a continued moist period in Thar desert. Lake pollen and other data support this inference. Onset of deglaciation weakened sand mobilization in the period.

Phase III (5000 to 3000 years BP). This is the most moist period with frequent floods. The rainfall is estimated to be between twice and thrice of the present. This period is, therefore, called as the climate optimum of the region with warm, humid and equable climate when great civilization of Mohanjo Daro and Harappa flourished at its peak and its decay set in toward the end of the phase – possibly by large fluctuations in the monsoon due to climate change from wet to the dry phase.

Phase IV (3000 BP to 18th century AD). This was the warm and dry period when the aridity had established once again and has continued almost unabated to the present with minor interdecadal and centennial scales of climate fluctuations in which somewhat wetter or drier regimes followed each other. This is supported by historical/archaeological evidence of rise and fall of post-Harappan cultures in the region like the painted grey ware (2800–2400 years BP) and the Rang Mahal culture (100 BC to 100 AD). The period between 600 AD and 1000 AD is inferred to be wetter and the period between 1400 and 1700 AD somewhat drier (Little Ice Age).

The interface between phase III and phase IV coincided with tectonic activity in the Aravallis region which brought changes in the courses of rivers Saraswati, Drishdawati, Sutlej, Ghaghar and Chambal. The drying out of the river bed of Saraswati and its feeding channels occurred and the glory of Saraswati was lost by the time of *Mahabharata*, i.e. 1500 BC or so[51]. Sand mobilization during this period correlates well with evidence about the desiccation of lakes[52]. Bryson and Baerries[53] suggest possibility of climate modification by human activity in the region during setting up of the natural dry phase which according to them would have led to more dust load and consequently aridity followed.

Local and regional dynamic studies on desert climate

Structure of summer-time heat low

In the recent years several special field experiments have been undertaken internationally and nationally in several countries to understand the dynamical structure of the atmosphere over desert regions. In India, the IITM Pune and the Gujarat Agricultural University, Anand are currently engaged with a land surface process experiment (LANDSPEX) over Gujarat region which is supported by the DST. Earlier in 1990, IITM carried out MONTBLEX[54] and a special observation on boundary layer processes was studied at Jodhpur. An elaborate experiment was carried out in the Arabian desert during the summer MONEX-1979 Programme[55]. The following results have emerged about the local/regional dynamics of the Thar/Arabian desert as a result of the above experiments and field studies carried out by the CAZARI scientists.

(i) Summer-time 'desert heat low' is a shallow cyclone at the surface which is replaced by a warm anticyclone above 1–2 km height.

(ii) Surface air temperature during most of the summer is 45–50°C and the surface soil temperature is higher by 10–15°C. Diurnal variation of air temperature is 20°C and surface soil temperature is 25 to 30°C. The soil cools rather rapidly with the onset of autumn and the winter minimum temperature reaches below the freezing point. Soil and air temperatures rise rapidly with sun rise and near-maximum temperature is reached by 1100 h and stays so for about 6 h in summer. By early night a surface inversion is set up even in summer.

(iii) The planetary boundary layer (PBL) is unique in summer (April to June) and is highly unstable for dry convection as super adiabatic lapse rate prevails. The thermal and wind-induced mechanical turbulence lasts for about 10–15 h during the day. The convective layer is up to 500 hPa (mixed layer height) in depth and the eddy transport of sensible heat is high from warm ground surface upward into the unstable PBL. This vertical advection of heat in the lower troposphere is about 3°C/day. During monsoon period (July and August) the PBL has sufficient moisture to realize conditional instability under favourable synoptic conditions.

(iv) The summer monsoon thermal 'low' undergoes quasi-periodic intra-seasonal oscillations on 5–7 and 10–15 day scales. These oscillations occur due to cooling above the mixed layer from an imbalance between the sensible heat flux divergence and the ground radiative heating. The dust load is mostly confined between the surface and mixed layer, which cuts off part of the solar radiation reaching the ground, leading to some decrease of temperature near the ground as well as reduction in vertical advection of sensible heat into the PBL. The heat law, thus, decreases in intensity (decay phase) and the mixed layer is somewhat stabilized reaching neutral phase from the convective phase.

(v) The dust layer plays a crucial role by absorbing incoming solar radiation and also by restricting outgoing thermal emission in the IR. Thus there is net radiative heating of 2–4°C/day in the troposphere above 700 hPa and net cooling of 6–8°C in the lower troposphere. This results in net cooling of the atmospheric column by 1–2°C at the top of the atmosphere. Radiation budget studies with *in-situ* and satellite-based observations confirm net tropospheric cooling on local and regional scales. As such, in order to sustain the desert heat low, it is necessary that energy is transported toward the desert by the large scale monsoon vertical circulation. This is accomplished by the moist ascent taking place over the convective regime centred over North Bay of Bengal and dry descent in the peripheral desert heat lows over the Thar and the Arabian deserts.

(vi) A two-cell structure in horizontal divergence prevails during the day in the troposphere in the heat low region with shallow convergence (at best up to 850 hPa) and divergence aloft up to mid-troposphere, topped by weak convergence in the upper troposphere. Maximum downward motion exists near the top of the mixed layer. During night time downward motion exists right up to the surface as the low level convergence is considerably reduced or even replaced by divergence.

(vii) Drag coefficient in the desert region[56] is 3 to 4 times higher than for the oceanic areas.

Bhide *et al.*[57] showed that at 500 hPa, heat sources and moisture sinks propagate westward on 10–15 day and 30–50 day scales from eastern part of the trough over the Bay of Bengal across the monsoon trough region. When a monsoon depression moves into the Rajasthan region, moisture is fed into the entire vertical column and tall clouds form, resulting in considerable rainfall over the region. The region of 'heat low' now becomes a region of heat source due to release of latent heat and moisture sink due to moisture convergence accompanying the weather disturbance. This is a transient phenomenon, which is replaced by the normal heat law pattern in about 3–5 days and the desert region again becomes a heat sink.

Regional and global and circulation cells and the Indo-Saharan desert climate

Two important vertical cells, viz. the Hadley cell in the south-north direction and the Walker cell in the east-west direction, have crucial links with the desert climate over the Indo-Saharan region. Hadley cell is primarily responsible for the year-round aridity as the desert regions fall in its downward limb. Even in summer monsoon season when the traditional Hadley cell has shifted north of India, the desert regions are mostly dry except during occasional travel of the monsoon disturbances to extreme western parts of India. This is as a consequence of two factors. Firstly, Das[58] showed the existence of a regional east-west vertical circulation in which deep ascent occurs over eastern India and the compensatory descent occurs over the Thar desert. This regional cell oscillates on sub-seasonal scale as discussed in the earlier paragraphs. Secondly a global scale Walker circulation prevails with ascent centred over eastern India and descent over the Indo-Saharan desert region in the west. This is supported by divergence at 200 hPa over the Tibetan high region and convergence in the adjoining mid-Pacific trough in the east and mid Atlantic trough in the west on the planetary scale. On interannual scale this global Walker cell along the summer ITCZ belt in NH is influenced by the ocean atmosphere coupled system in the equatorial eastern Pacific, commonly known as the ENSO phenomenon. During the warm phase of the ENSO, the Tibetan high at 200 hPa is displaced more eastward. A zone of convection migrates eastward from Indonesia to the central Pacific and the large-scale convective activity is decreased over India and adjoining tropical belt. This results in major monsoon drought over SE Asia, India, Somalia and sub-Saharan regions.

Numerical atmospheric general circulation experiments on desert climate

Numerical experiments

ACGMs have been extensively used in the last two decades to understand the process of desertification on regional scale. The premise for such studies is on three approaches, all of which have links with the energy balance principle, as applied to deserts. Deserts are regions of net energy deficit, because of high albedo, high long-wave emissions, high sensible heat and low latent heat contribution in the desert–atmosphere system. Otterman[59] and Charney[60] proposed hypothesis for the desertification which was based on the increase in albedo of the deserts by human activity such as overgrazing and loss of vegetation cover with consequent more radiative energy loss and decrease in temperature of the desert environment and reduction in convective overturning, resulting in enhancement of the desert system. The second hypothesis was tested by the numerical experiments of Walker and Rowntree[61], Shukla and Mintz[62] and several other workers since then. It is based on the vegetation–atmosphere interaction involving the role of soil moisture and evapotranspiration in modulating the energy balance, rainfall, temperature and moisture fields. A third approach was suggested by Sud and Smith[63] and Sud *et al.*[64] which rested on changes in surface roughness due to decrease in vegetation affecting the surface momentum balance and hence the efficiency of heat and moisture transports in the desert–atmosphere system.

All the three approaches involve biogeophysical mechanisms and are linked with land–vegetation–atmosphere or the land surface processes. Although some observational studies support the above approaches, they can best be tested by employing controlled and anomaly GCM experiments through sensitivity studies. As such a spate of such experiments on all the hypotheses have been performed by different numerical modelling groups since the first work by Charney *et al.*[65] and Chervin[66], using different parametrization schemes for the land surface processes, some of which even include separate biospheric models. Good reviews on the subject are provided by Dickinson[67], Rowntree[68], Sud *et al.*[69] and Dirmeyer and Shukla[70]. Many numerical modelling studies have been carried out to understand regional droughts such as Sahellian, north American Great Plains, Australian and South African droughts on the above three approaches since 1970s. In a review of 11 such studies, Mintz[71] concluded that soil moisture had a stronger impact on model-simulated climate than did albedo or surface roughness. Some of the models have been used to understand the role of biogeophysical processes on the monsoon rainfall in western Indian desert and the results are mixed[64,72,73]. This may be, among other factors, due to the fact that the numerical models have yet to produce an acceptable simulation of the monsoon rainfall of India and hence at this stage may not be very appropriate tools for sensitivity studies on monsoon. Recently Fennesey and Shukla[74] have provided a review of the role of soil moisture anomaly studies on precipitation and temperature anomalies in the mid-latitude belt of USA.

There is a variety of observational evidence supporting and even contradicting the expectations based on biogeophysical feedbacks. Recent observational studies have pointed to reduced importance of albedo feedback on desertification and have challenged Charney's hypothesis by showing that surface warming instead of surface cooling occurs during prolonged droughts. Besides they also claim that Charney's hypothesis does not explain synchronous occurrence of major droughts over several regions such as in Sahel, Kalahari, Australian and Thar deserts. It also does not explain the termination of such droughts. Thus albedo model is now considered as interesting but unproven. On the other hand, soil moisture mechanism for desertification by bringing changes in the hydrological parameters through vegetation–atmosphere interactions, has gained importance. The major factor for the synchronous occurrence of droughts on inter-annual and inter-decadal scales is considered to be the coupled ocean–atmosphere ENSO phenomenon. However, it cannot be ignored that human activity has pronounced influence on the local scale desert climate through accelerated soil erosion, deforestation and loss of vegetation and soil organic matter by over-grazing and over-exploitation of the soil resource. In areas without irrigation facilities, desertification can lead to increased temperature and reduced rainfall. Irrigation can reverse these effects, as it has happened in the Indira Gandhi Canal command area, but it is producing increased salination of the soil.

Numerical experiments on expansion of desert climate and global climate

As deserts are important sub-component of the global climate system, there is a concern that expansion of deserts may impinge on the global climate. Observational evidence is mounting that desertification has been occurring extensively over the world for the last several decades[75–77]. In order to specifically address the problem of enhancement in desert areas and their possible impact on regional global climate, Dirmeyer and Shukla[78] have used a GCM and the realistic land surface properties with present day extent of deserts (control run) and doubling their extents (anomaly run) over all the continents. The deserts were extended by altering the land surface properties and type of vegetation over the present day deserts and their margins. The double desert case represents an extreme scenario of desertification which might ensue as a result of natural and anthropogenic causes. The results of the control and anomaly runs for 10 years were compared and showed the following aspects prominently:

(i) Significant cooling over much of the tropics and sub-tropics and the cooler air in the double desert case covered an area nearly six times the size of the current deserts. This impact is, therefore, global in nature.

(ii) Rainfall generally, but invariably, decreases over desertified areas. Over Africa the decrease is largest (20 to 60%). Over the leading edge of the Indian monsoon region such as the Gangetic plains and adjoining Myanmar and China significant decrease in rainfall (about 10%) is also noticed. Some increase in rainfall is noticed over Peninsular India, Sri Lanka and Malaysia. Similarly evapotranspiration anomalies are larger over Africa than over South Asia. Lower response over South Asia is perhaps due to the fact that this core region of the monsoon is influenced by warm SST in Western Pacific and Bay of Bengal regions as well as by the elevated heat source over the Tibetan region.

The results of this experiment more or less followed Charney's hypothesis with regard to cooling over the deserts. The experiment of Dirmeyer and Shukla[78] no doubt points to potential danger of decreased rainfall over much of the tropics and sub-tropics in response to doubling of the present extent of the deserts.

Concluding remarks

We have discussed many aspects of desert climate based on the work of many researchers. Desert climate is harsh for human habitation as these ecosystems are highly responsive to climate variability. However, man has lived in several arid and semi-arid areas for thousands of years in harmony with nature, particularly in the Thar desert which is unique for it lies on the western margin of the Indian Summer Monsoon System and where the population is fairly high. Population pressure and increasing exploitation of the vegetational and soil resources in desert lands are raising serious doubts among experts whether nature can sustain such over-exploitation resulting from human greed. As desertification and desert climate are intimately linked, several science and technology measures would benefit sustainable development of the arid and semi-arid regions and some of them are:

(a) Provision of natural resource information to local people and policy makers. For India such an information system is being developed by DST and the Department of Space in collaboration with NRSA.

(b) Enhancement of regional climate monitoring network.

(c) More numerical modelling studies and their coupling with empirical and observational studies such as land surface field experiments.

(d) Application of seasonal monsoon forecasting for contiguous arid and semi-arid regions separately rather than the present system which addresses India as a whole.

Desert climate is an integral sub-component of the global climate. However, if desertification is allowed to go unabated, numerical modelling studies point to a scenario that it would impact on the climate of regions farther away from deserts themselves. This would be an unfortunate event for the modern world civilization which is based on intensive development culture and over-consumption. There is a silver lining that the problems posed by desert and threatened desert margin climates can be tackled adequately by combining modern science and technology with the traditional rational knowledge of the inhabitants of the regions, who have learnt to live for many generations in harmony with their rather harsh natural environment. They have used its fragile resource on sustainable basis till now with wisdom born out of experience. Science and technology can surely help them to do it for future too.

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Role of remote sensing in resource management for arid regions with special reference to western Rajasthan

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The arid region of western Rajasthan is about 20.87 Mha covering 85 blocks of 12 districts of Rajasthan which constitutes 61% of the state. About 38% of the total population of the state with a density of 84 persons per sq km area lives in the arid region of Rajasthan. The constantly increasing human and livestock population is putting tremendous pressure on the available natural resources. The major problems of the region are: scarcity of water, frequent droughts, land degradation, deteriorating pasture lands and advancement of desert. Therefore, it is essential to explore newer sources of resources as well conserve the existing natural resources. Optimal management of natural resources is of utmost importance in today's context to increase the production of food grains, fodder and develop water resources for meeting the demand. Remote sensing provides timely, reliable and spatially comprehensive data on various natural resources for effective planning, implementation and monitoring. Some of the natural resources problems of the western Rajasthan, use of remote sensing techniques and role of current and future Indian remote sensing programme are highlighted in this paper.

ARID zone in India covers about 39 million hectare (Mha) area constituting about 12% area of the country. It covers 32 Mha as hot and 7 Mha as cold arid zone. Twelve districts in western Rajasthan covering 20.87 Mha fall in the hot arid zone, constituting around 61% of the total area of the state. These districts are Barmer, Bikaner, Churu, Ganganagar, Hanumangarh, Jaisalmer, Jalore, Jhunjhunun, Jodhpur, Nagaur, Pali, and Sikar and together constitute 85 blocks. According to the 1991 census about 38% of the total population of the state with a density of 84 persons per sq km area lives in the arid region. The livestock population is around 25 million (1987–88) with a density of 120 per sq km. This makes it one of the most densely populated deserts of the world. The constantly increasing human and livestock population is putting tremendous pressure on the available natural resources.

The western Rajasthan has typical characteristics. They are (i) mean annual rainfall of about 310 mm, (ii) about 50% of the region has plain lands without stream network, (iii) about 30% of the region has slopy lands with stream network mainly in the Luni basin, (iv) about 20% of the region is covered under Indira Gandhi canal command area.

The main problems of the region are: (i) low and erratic rainfall, frequent droughts, (ii) scarcity of water, (iii) available groundwater resources is also not sufficient since it is brackish/saline at many places and found at greater depths with low discharge, (iv) soil is less productive, (v) water logging problem in Indira Gandhi Nahar Project areas, (vi) wind erosion causing high erodibility of sandy soil, (vii) deteriorating pasture lands and (viii) advancement of desert.

Remote sensing is the science of making inferences about material objects from measurements, made at distance, without coming into physical contact with the objects under study. In this context, any force field – gravity, magnetic or electromagnetic, could be used for remote sensing, covering various disciplines from astronomy to laboratory testing of materials. However, currently the term remote sensing is used more commonly to denote identification of earth features by detecting the characteristic electromagnetic radiation that is reflected/emitted by the earth surface.

Satellite remote sensing provides synoptic view, enabling one to understand the inter-relationship between various features observed. In addition, remote sensing satellites cover the same area repetitively, making it possible to understand and monitor changing physical processes. Availability of data at different spatial resolution provides information at macro level and micro level. Microwave remote sensing provides all weather capability. Thus satellite remote sensing provides timely, reliable and spatially comprehensive data on various resources for their exploration, monitoring and effective management.

The data received from the satellites can be analysed using computer or by visual interpretation. For visual interpretation the data is converted into photographic images. The photographic images are usually generated using green, red and near infrared (NIR) assigning blue, green and red respectively. This is called False Colour Composite (FCC) since it does not depict true colour, as the information of the blue band is missing. Since for the vegetation the reflectance in NIR is higher, the vegetation appears red in the FCC. Figure 1 shows the imagery of western Rajasthan produced by the IRS-1C WiFS camera, with the district boundaries overlaid. It depicts vegetation biomass in red colour wherein good vegetation is seen in Ganganagar, part of Hanumangarh, east Nagaur, Jhunjhunun, Sikar, Pali and Jalore districts. Medium vegetation is seen in central part of Jaisalmer and west part of Jodhpur districts. Low vegetation is seen in part of Bikaner, east Barmer and Churu districts. No vegetation/dry area are in parts of Jaisalmer, Barmer, Bikaner, Churu and Hanumangarh districts.

Table 1 gives some of the major problems in the region of Rajasthan and possible applications of remote sensing to help in evolving suitable strategy for their management. The details of application of remote sensing in tackling some of the natural resource problem areas are discussed here.

Water resources

The western Rajasthan faces acute shortage of potable water and village women have to fetch water from many kilometers away to meet domestic requirements. Sixty to sixtyfive per cent of western Rajasthan has saline to highly saline groundwater with the TDS (total dissolved salts) content ranging from 2000 ppm to 20000 ppm. In addition to problem of obtaining potable water, deterioration of land due to the use of poor quality groundwater for irrigation is causing serious concern.

Remote sensing aids quick identification of groundwater prospective zones. Some of the indicators of groundwater prospective zones are lineaments, lineament intersection, palaeochannels and valley fills. During the drought of 1986, hydrogeomorphological maps on 1 : 250,000 scale for the entire state using Landsat TM imagery were prepared. These maps show hydrogeomorphological units and the related groundwater prospects. A typical hydrogeomorphological map of Shahbad Tehsil of Baron district in Rajasthan is given in Figure 2.

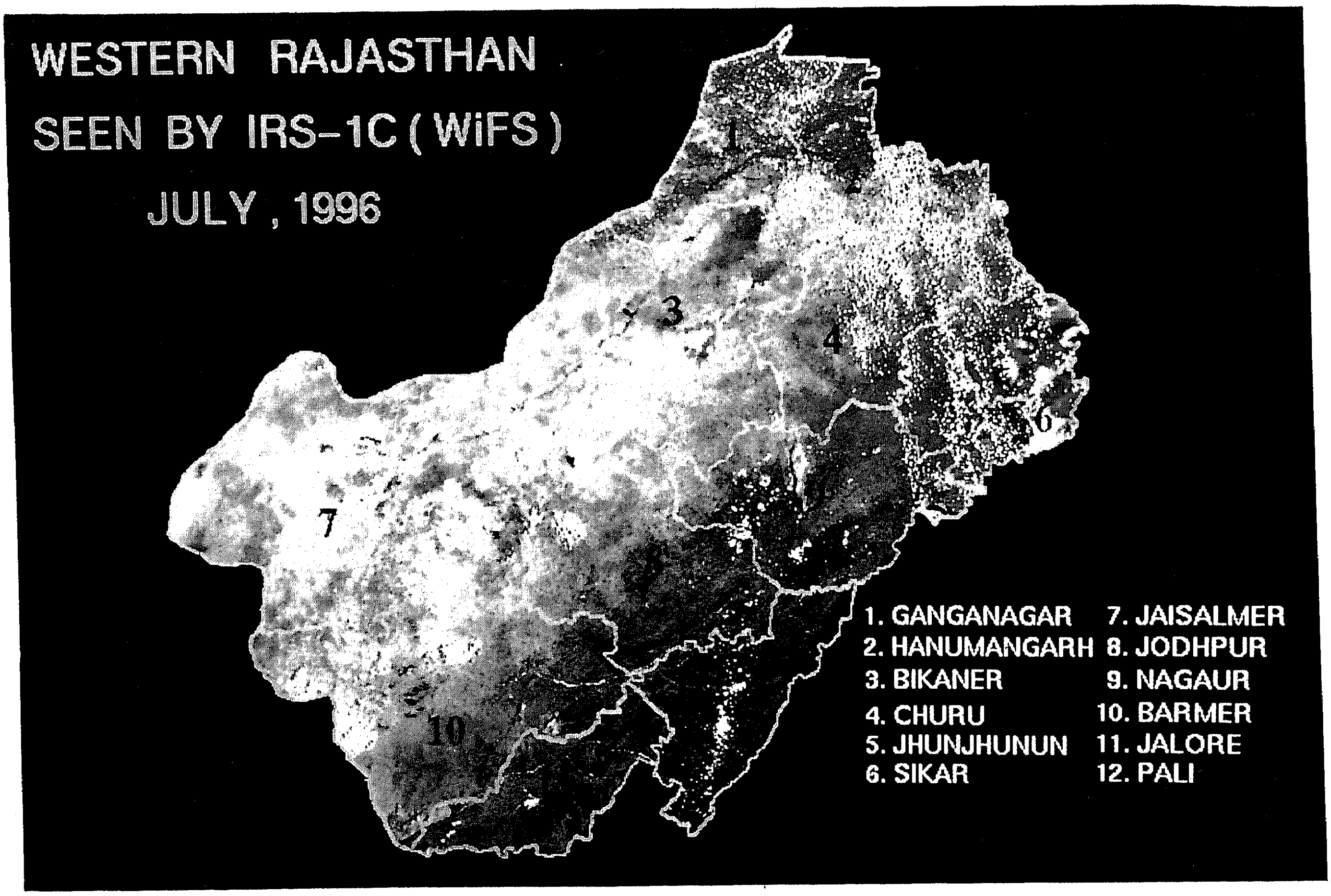


Figure 1. IRS-1C WiFS view of western Rajasthan with district boundaries overlaid.

Table 1. Major resource management problems in arid region of Rajasthan

Problems	Remote sensing potential
Scarcity of water	Identification of groundwater prospective zones, Sites for recharge structures
Frequent droughts	Assessment of drought conditions through NDVI
Advancement of deserts	Monitoring of sand drift/movement
Land degradation	Monitoring wastelands, Water logged area, Land use/cover, Impact of mining
	Prioritize erosion prone area for soil conservation measures
Deterioration of pasture lands	Grassland inventory, condition assessment and monitoring

The map shows spatial extent of various hydrogeomorphological features. Good to very good groundwater prospects are represented by valley fills, lineaments and buried pediment in sandstone. These maps provided the 'first cut' information for carrying out follow-up hydrogeological and/or geophysical measurements for pinpointing sites for exploratory drilling. Detailed mapping for Jaisalmer and Bikaner districts on 1 : 50,000 scale has been carried out using IRS LISS-II and partially SPOT images. Currently under the Integrated Mission for Sustainable Development, detailed action plans for water resources development like sites for water conservation and harvesting have been generated for many districts in the western Rajasthan, where the satellite data and field data have been integrated. Efforts have also been

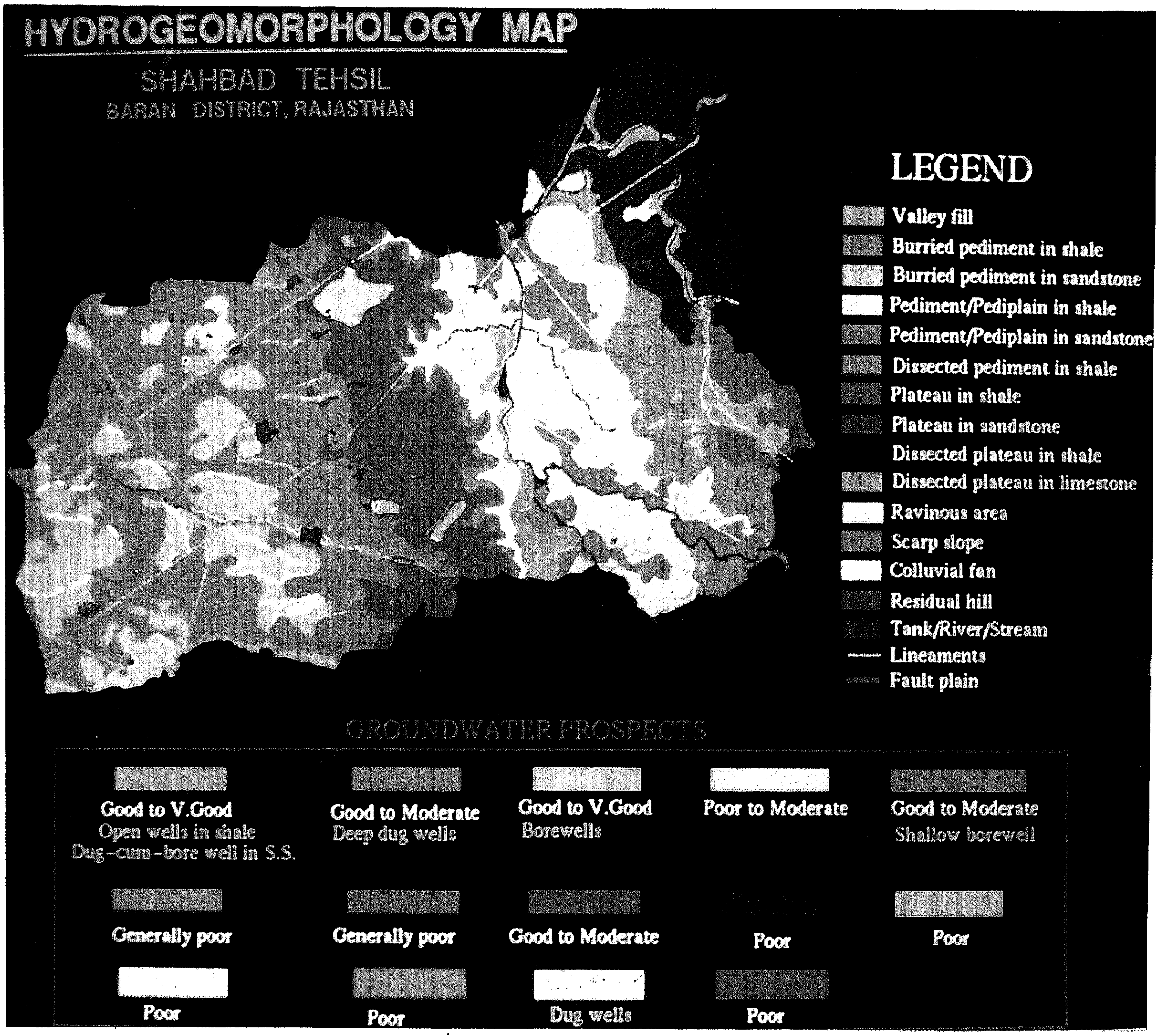
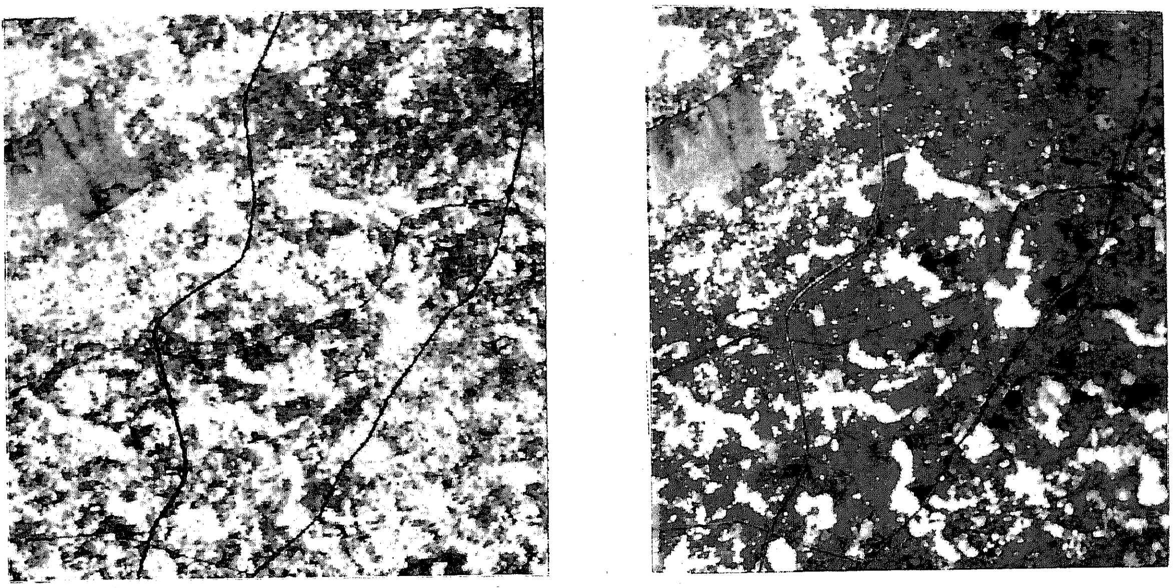


Figure 2. A typical hydrogeomorphological map showing groundwater prospects in Shahbad tehsil, Baran district, Rajasthan.



MSS October 1975
Cropped area: 36073 ha
Waterlogged area: Nil

IRS LISS II October 1995
Cropped area: 57208 ha
Waterlogged area: 6885 ha

Figure 3. Impact of IGNP: increase in cropped area (red tone) and increase in water logged area (black and bluish tone) in October, 1995 image as compared to October, 1975 image.

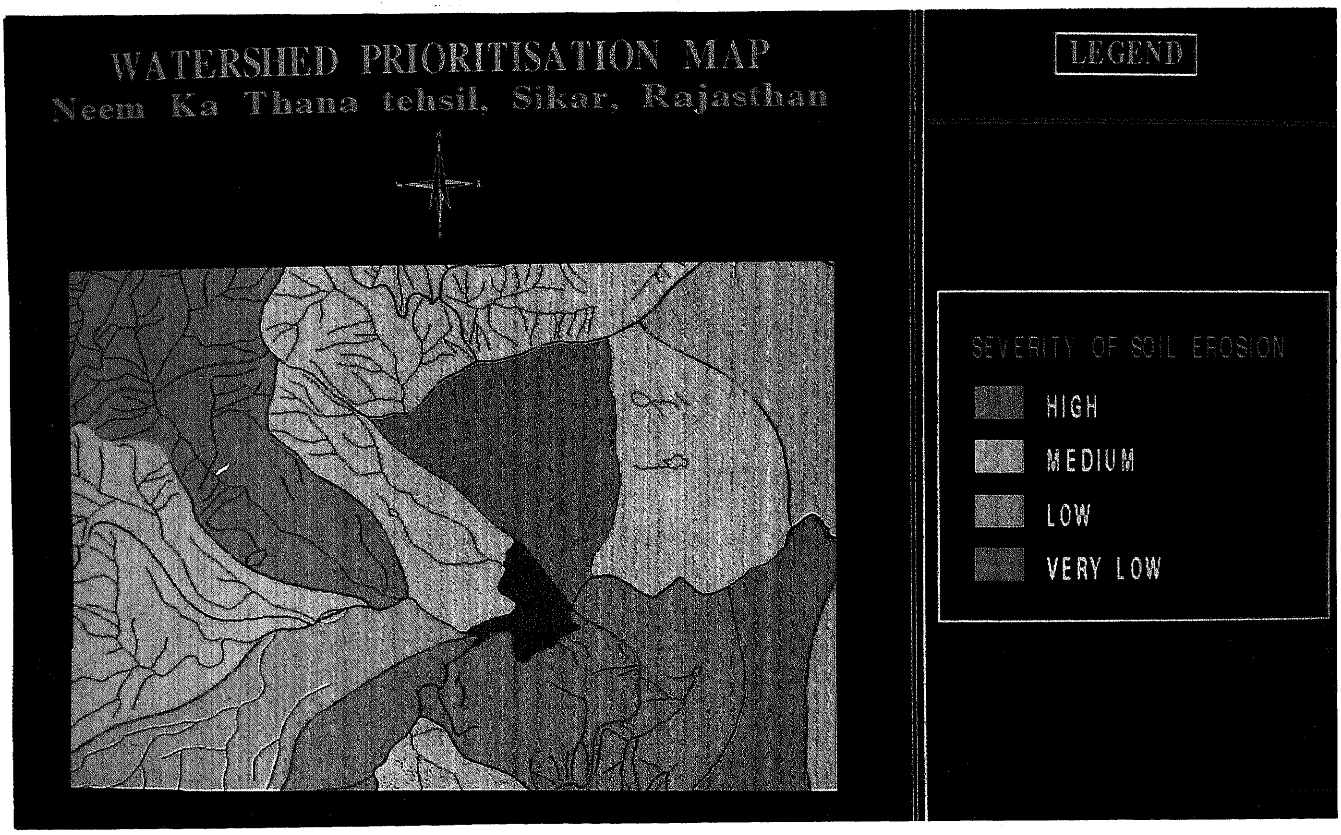

WATERSHED PRIORITISATION MAP
Neem Ka Thana tehsil, Sikar, Rajasthan
N
LEGEND
SEVERITY OF SOIL EROSION
HIGH
MEDIUM
LOW
VERY LOW

Figure 4. Watershed prioritization map showing severity of soil erosion in Neem ka Thana tehsil, Sikar district, Rajasthan.

made to explore potentials of radar (ERS-1 SAR) data in identification and mapping of buried channel and relict valleys near Jaisalmer for groundwater prospecting. Also a synergistic methodology of integrating optical with microwave data to reconstruct the palaeo drainage network of western Rajasthan is being attempted.

To sustain the groundwater potential it is essential to recharge the groundwater. The recharge is achieved primarily by three methods, viz. i) spreading, ii) induced recharge, iii) injection. Remote sensing provides useful inputs for siting various recharge structures, through thematic maps on hydrogeomorphology, land use/cover, surface water bodies, slope, etc. The main guiding factor is location of a permeable zone. Some of the indicators, which can be deciphered from the remote sensing images, are crossing zones of dykes over streams, low drainage density, higher lineaments intensity, confluence of channels, valley fills, palaeochannels, etc.

Drought assessment and monitoring

Drought in western Rajasthan is a regular phenomenon. Generally, mild and moderate agricultural drought occurs in the entire region, while the Jaisalmer region experiences severe drought conditions. The climatologists have estimated that the drought occurrence probability in western Rajasthan is less than 20%, implying that, on an average, one in every five years is expected to have large rainfall deficit. The sand and dust movements generally assume serious proportions in the years following the drought year, when the vegetation cover is minimum. Sand flux rates of up to 150 kg per square meter are common during the peak of dust storm activity. Generally May and June are the periods of strong wind when sand movement causes problems for human settlements and transportation networks. The average wind speed during June is more than 25 kmph in the western part, while in the eastern part it is between 10 and 15 kmph. These natural climatological conditions have impact on acute water shortage and land degradation.

Satellite data has proved to be extremely useful in monitoring drought. Starting from mid 1989, the National Remote Sensing Agency (NRSA) has been issuing fortnightly drought bulletins based on the analysis of the NOAA–AVHRR data for the entire country including Rajasthan. The bulletin contains maps on vegetation index, comparative greenness and district-wise assessment of the drought like normal, mild, moderate or severe. In the present day context IRS-1C data with high spectral/spatial resolution can even provide village level severity assessment and thereby determine the quantum of relief assistance and crop insurance.

Advancement of desert

The western Rajasthan suffers from destabilization of dune systems due to high wind erosion, excessive grazing by livestock and cattle coupled with other activities like road building, canal excavation, quarrying, ploughing besides deforestation. It is estimated that nearly one third of the cultivated lands is severely affected by the problem of sand drift, while another one-fourth is moderately affected. The mean soil loss in the deeply ploughed fields for example in an unusually windy year of 1985 was estimated to be 2837 tonnes/ha (ref. 1). The spread of desert towards east and north east has endangered the productivity and fertility of soil cover. A regular monitoring is therefore required for the above activities so that suitable remedial measures can be suggested.

Change detection on the fringe of the desert region in Rajasthan has been carried out using multi temporal satellite data and digital analysis techniques. This has brought out that the desert is spreading through something like twelve large size gaps in the central and northern Aravalli hill ranges. This study thus speculates that an area of about 160 km^2 every year is engulfed by the advancing desert. Measures to check the spread of desert have been suggested[2].

Under a joint study by CAZRI and RRSSC, Jodhpur a number of sites have been studied to identify the stability of sand dunes using multi-temporal satellite data which causes shifting of sand.

Grassland inventory and monitoring

The region receiving mean annual rainfall of 200 mm and less is incapable of supporting agriculture on its own. It can sustain animals on its natural vegetation comprising of shrubs and grasses. Animal husbandry replaces agriculture in this region, which is crucial to the economy of the region. Therefore, the natural grasslands are one of the most precious natural resources. Amongst the other grasses, Sewan (*Lasiurus sindicus*) is the dominant perennial grass. There are nearly six million ha of Sewan-dominated open pasture lands. These grasslands are being degraded either due to impoverishment of the useful species or indiscriminate grazing. Nearly 24 and 44% of the pasturelands are in desertified and highly degraded states respectively.

Satellite data have been used to carry out inventory and monitoring of the areas under Sewan grass. Using multi temporal IRS LISS-II data of 1988 and 1989 and image differencing techniques, areas under four categories of Sewan grasslands in Jaisalmer, Bikaner and parts of Jodhpur district could be estimated and a reliable inventory of this crucial resource could be made.

Locust warning and surveillance

Locust breeding is one of the major problems in western

Rajasthan as it poses threat to the standing agricultural crops and plantations. Moist sandy soil of the desert and flush of desert vegetation during monsoon creates optimal conditions for fast locust breeding and development.

In the later part of 1989 there was a sudden build up of locust swarms from Pakistan to India. The Locust Warning Organization of the Directorate of Plant Protection, Govt of India used IRS and NOAA data to generate five green biomass levels within the sparse desert vegetation. Multidate imagery helped to monitor and launch selective control measures in an area of 200,000 km^2 (ref. 3).

Land degradation *vis-à-vis* IGNP

In the canal command area under Indira Gandhi Nahar Pariyojana (IGNP), Stage I a mean rise in water table of more than one metre per year has been recorded. This is leading to the problem of land degradation as the productive land is getting converted into water-logged and salt-affected areas.

Environmental impact of the Indira Gandhi Canal in the command area has been monitored using multidate IRS LISS II and NOAA–AVHRR data. The analysis of satellite data helped in monitoring land use changes, mapping and assessing the progress of afforestation programme along the canal network, monitoring the extent of waterlogged areas, inventory of irrigated crop, assessing crop water demand and monitoring crop growth patterns. Figure 3 shows the Rawatsar area satellite imagery of October 1975 and October 1995. The distinct red colour in October 1995 image clearly depicts increased cropped area as compared to October 1975 image. The waterlogged areas are seen in 1995 image as black and bluish patches. The cropped area has increased by more than 50% from 1975 to 1995. However, waterlogged area has also increased due to excess irrigation and poor drainage. Thus remote sensing can be used to monitor the change during every cropping season and the information is vital for proper land use planning and corrective measures to be taken.

Wasteland inventory

Large areas in the arid region are under wasteland category. The development of wastelands not only helps in improving the land productivity and socio-economic conditions of the region but also helps in re-establishing the ecological balance.

Under the National Wasteland Mapping Project, wasteland mapping at 1 : 50,000 scale using Landsat TM data has been carried out for three districts namely Churu, Jodhpur and Pali. Various categories of wasteland like gullied/ravinous land, upland with or without scrub, land affected by salinity/alkalinity, sandy areas, mining-industrial wasteland, under-utilized/degraded notified forest land, degraded pastures/grazing land, barren rocky/stony wastes/sheet-rock areas, and steep sloping areas have been mapped. It has been estimated that about 60.9, 30.6 and 26.6% of the total area of Churu, Jodhpur and Pali districts respectively is under wasteland. Depending upon the nature of the wasteland categories at village level, reclamation measures have been suggested.

Soil erosion studies

Soil erosion seriously affects the productivity of land. Life of a reservoir is dependent on the soil erosion in the catchment areas. It is essential to characterize the watershed according to intensity of erosion-prone areas, so that soil conservation measures can be taken on priority in those areas which are highly prone to erosion. For this purpose thematic maps such as land use/cover, geomorphology, slope and soil are prepared using remote sensing and conventional data. Integrating these maps and suitable models, it is possible to categorize watersheds into various severities of erosion. A classified image showing severity of soil erosion for Neem Ka Thanna tehsil of Sikar district is shown in Figure 4. High priority watersheds, shown in pink colour, represent hilly areas having steep slopes and degraded forest lands. Similar work has also been carried out for Kishanganj, Shahbad, Devli and Nadoti blocks of Baran, Tonk and Sawai Madhapur districts respectively.

Agroclimatic-zone based land use planning

A systematic district-wise land use mapping on 1 : 250,000 scale has been carried out using IRS LISS-I data. These maps show Level I and II categories. Using two-date imagery, acreages under kharif and rabi crops have been estimated. The information thus generated is being extensively used for agroclimatic-zone based land use planning. Presently 49.4% of the total area is under cultivation, 35.5% area is under cultivable wastes and fallow lands, 4.1% area is under permanent pasture, 1.8% area is under forest and 9.2% area is either barren or settlement[1].

Mineral prospecting

The occurrence of minerals is not random, i.e. mineralization is always confined to specific geological environments and various guides for mineral prospecting are utilized. Satellite data indicates, for e.g., i) zones of crustal weakness, e.g. faults, fractures, joints, folds, shear zones, etc. which serve as passage ways for mineralizing solutions, ii) tonal anomalies – alteration zones, vegetation anomalies, etc. and, iii) major rocks types and landforms.

Important industrial minerals and rocks occurring in the western Rajasthan are the building stones (like marble, limestone, sandstone and granite), limestones of cement, flux and chemical grades, various types of clays for ceramics, common salt, bentonite and Fuller's earth, gypsum/gypsite and phosphorite, lignite and wolframite.

Although there has been limited use of satellite data for mineral exploration it has been observed that gypsite (earthy porous gypsum aggregate with sand, clay and calcareous matrix) occurs in low-lying playa deposits and in palaeochannels, which can be mapped using satellite data. Rajasthan accounts for about 90% of the total production of gypsum in the country. In the western parts of the Jaisalmer district the Khuiala and Bandah limestone deposits occur. It has been estimated that about 2500 million tonnes of chemical and flux grade limestone occurs in the region. In this region recently ERS-1 SAR data could detect shallow sand buried limestone deposits. The structural and geomorphological information in the Jaisalmer district has provided useful clues to the oil and natural gas exploration.

Integrated mission for sustainable development

Realizing the importance of adopting an integrated approach, and recognizing the mutual inter-dependencies of natural resources, the Department of Space, at the behest of the Planning Commission, Govt of India has taken up a major programme 'Integrated Mission for Sustainable Development (IMSD)', along with various state and central government agencies. Participation of voluntary agencies and the local people is an important component of this programme. Under this mission, it is proposed to undertake remote sensing-based integrated land and water resources studies for 174 problem districts of the country. The major goal of this mission is to generate locale-specific plans by integrating natural resources information generated from satellite data in conjunction with socio-economic data to meet the needs of the local people for sustainable development of the region. The goal when translated into executable tasks results in the following:

i) Analysis of socio-economic, demographic data of the region to find the felt/perceived development needs of the region.
ii) To generate various thematic maps on natural resources and integrate them to identify homogeneous units of land parcels for suggesting measures for soil conservation, water resources development, agriculture/horticulture, and fodder development.
iii) To critically assess the existing infrastructure of the region and to arrive at development schemes (suitable sites for location, alternate strategies, etc.).

The Regional Remote Sensing Service Centre (RRSSC), Jodhpur along with State Remote Sensing Applications Centre (SRSAC), Central Arid Zone Research Institute (CAZRI), and other Non-Government Organizations (NGOs), is carrying out IMSD studies in ten districts, viz. Barmer, Bikaner, Churu, Nagaur, Jalore, Jaisalmer, Jhunjhunun, Jodhpur, Sriganganagar, Sikar and Pali of western Rajasthan and Jaipur, Dausa, Ajmer, Bharatpur, Sawai Madhopur, Banswara, Dungarpur in east and south Rajasthan. Besides this, eleven blocks under special IMSD study have been completed. These are Kishanganj, Shahbad, Ramganj, Mandi, Sangod, Kherwara, Garhi, Nadoti, Devli, Dag, Dungarpur and Masuda.

Indian Remote Sensing Programme

Indian Space Research Organization (ISRO) successfully developed and launched the operational first generation remote sensing satellite IRS-1A in March 1988 and the follow-on IRS 1B in August 1991. These satellites carried two imaging payloads operating in four bands in the 0.45–0.86 μm region. One of the payloads (LISS-I) has the geometric resolution of 72.5 m and a swath of 148.48 km and the other camera LISS-II has a geometric resolution of 36.25 m. Two LISS-II cameras were displaced laterally so as to provide a combined swath of 145.48 km with an overlap of 3 km (ref. 4). A third operational remote sensing satellite IRS-P2 was launched in October 1994 using the Polar Satellite Launch Vehicle (PSLV) developed by ISRO. IRS-P2 carried a modified LISS-II camera.

Data from these remote sensing satellites have been extensively used for various applications towards national development. Starting from field experiments to district level studies, currently a number of projects are being carried out covering the entire/most part of the country on an operational mode. Groundwater targetting, Regional Geological Mapping, Flood Mapping, Forest Mapping, Land Cover Mapping, Wasteland Mapping, Snow Area Mapping, Crop Production Estimation, Watershed Characterization, Coastal Zone Mapping, etc. are some of the national level projects taken up. These have been taken up in 'mission-mode' under the umbrella of a programme called Remote Sensing Applications Mission (RSAM). The Integrated Mission for Sustainable Development (IMSD), wherein various themes using remote sensing and socio-economic and other data are integrated to find out optimum developmental plan opens new vistas to apply remote sensing technology for practical application.

The IRS-1C launched in December 1995 carries three distinct and mutually complementing imaging payloads which enhance the capabilities of IRS-1C as compared to IRS-1A/1B in terms of spatial, spectral and temporal resolutions. Another remote sensing satellite IRS-P3 was launched on March 1996 with PSLV carrying a Wide Field Sensor similar to IRS-1C, with an additional band in SWIR and an ocean colour monitor, developed by DLR (Germany) for earth observation.

The IRS-1C has a PAN camera with 5.8 m resolution, a multispectral camera (LISS-III) with 23.5 m resolution in visible and near infrared region and 70.5 m in the shortwave infrared region. The PAN camera covers a ground swath of 70 km which is steerable up to ±26° from nadir in the across track direction. This off-nadir viewing provides the capability to acquire stereoscopic pairs from two different orbits and an ability to revisit any given site with a maximum delay of five days. Currently the IRS-1C, PAN camera is the highest spatial resolution civilian earth observation system.

A Wide Field camera (WiFS) has a spatial resolution of 188 m and covers a swath of 804 km. This wide swath coverage results in a repeatable observation of the same ground location after every 5 days. The WiFS operates in the B3 and B4 spectral bands of LISS-III.

Application potential of IRS-1C

The payloads of IRS-1C complementing each other for earth viewing have immense application potential which was hitherto not possible. Some of the unique advantages from the applications point of view are:

- Monitoring vegetation, snow and water body more frequently (5 days) and mapping them at regional scale
- Improved identification/discrimination of vegetation types/crops in view of higher spatial resolution (VNIR 23 m) and SWIR band
- Moisture stress detection especially by the use of SWIR band
- Large scale mapping using merged LISS-III and PAN data
- Stereo capability and frequent viewing using PAN
- Availability of data at different spatial resolutions from a single platform (acquired simultaneously) suitable for multistage/sampling approach.

Using the data available so far from IRS-1C, a host of applications have been demonstrated.

Future Indian Remote Sensing Satellites

IRS-1B, IRS-P2, IRS-P3 and IRS-1C are presently in use providing information on earth resources to various user agencies on an operational basis. IRS-1D, which is similar to IRS-1C, is planned to be launched in 1997. Other approved space systems for future include Oceansat-1 carrying an ocean colour monitor and a multifrequency radiometer, a cartographic satellite with fore and aft viewing with about 2.5 m resolution and a satellite system tuned to meet many of the needs for agricultural applications.

Future systems being conceived include Oceansat-2 carrying various sensors for ocean applications such as scatterometer, altimeter and radiometer, satellites for climate studies and high resolution imaging systems for updation of large scale maps.

All these missions providing data at higher spatial and temporal resolutions along with the technological advances made in processing and modelling techniques including GIS should be revolutionizing the field of remote sensing applications in the country. It is for us, the user community of scientists, policy and decision makers, planners and implementation level officers of various government and non-government agencies to make full use of this opportunity for national development.

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Isotope hydrology studies on water resources in western Rajasthan

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Environmental isotope studies in the Jalore and Barmer areas show that deep groundwaters have depleted ^{2}H and ^{18}O compared to the present day precipitation and have negligible tritium. Radiocarbon levels indicate groundwater ages ranging from 2400 to 7400 BP. Shallow groundwaters, on the other hand, show bomb tritium indicating some component of modern recharge. Studies on the effect of the Indira Gandhi canal on groundwater environment helped in differentiating the areas waterlogged by canal seepage and those affected by return flow of irrigation. A study on the buried channel of the legendary 'Saraswati' river in Jaisalmer district indicates shallow waters are old with ^{14}C content of 54.9 pMC to 58.8 pMC (uncorrected ages: 4950 to 4400 BP) with a velocity of about 20 m/a.

ARID zones pose special problems to hydrologists anywhere in the world. Conventional techniques have methodological constraints[1]. Some are: a) paucity of rainfall data due to irregular distribution in time and space and frequently disastrous and ungaugable storm events, b) untested extrapolation of the estimates of potential evapotranspiration to arid regions, and c) limitations on the use of geoelectric profiles due to dry shallow horizons and deep saline formations.

On the other hand, experience in the applications of isotope techniques in India and elsewhere indicated that the techniques are better suited to arid zones than to any other geographical situation. The reasons are: 1) The isotopic composition (^{2}H and ^{18}O) of rainwaters depends on the temperature of condensation, altitude and intensity of rainfall. This results in different isotope signatures for different rain events, particularly in the arid and semi-arid regions. 2) Evaporation, unlike condensation, causes kinetic fractionation of ^{2}H and ^{18}O and provides an identifiable isotopic characteristic. 3) Paleowaters from earlier pluvial episodes have depleted ^{2}H and ^{18}O, insignificant tritium and low levels of ^{14}C in dissolved bicarbonates. Radiocarbon helps in the age-dating of paleowaters.

In addition to the above, it is possible to tag a layer of soil moisture in the unsaturated zone with a suitable radioactive tracer like tritiated water or ^{60}Co labelled $K_3Co(CN)_6$ (ref. 2) and follow its movement to evaluate rainfall recharge.

Recharge to shallow aquifers is the subject of great interest to groundwater management in arid areas since modern recharge to deep groundwater is relatively rare.

Isotopes help in answering such questions as

- Is there modern recharge?
- If so, what is the recharge process; direct infiltration of rainwater, contribution during flash floods, return flow of irrigation, etc.?
- If not, when was the aquifer last recharged?

Some typical isotope studies carried out in western Rajasthan are discussed briefly in the following sections.

Jalore district

An environmental isotope study was undertaken to investigate groundwater recharge in the Jalore area of Rajasthan. The study area located in Jalore district of southwestern Rajasthan (Figure 1) is arid and receives mean annual rainfall of 380 mm. The area is covered extensively by alluvium. Presence of sand dunes is a common feature in the area, especially in the western part of the study area. The surface drainage consists of Sukri river, a tributary of the Luni river system, which is ephemeral in nature and flows towards southwest, i.e. towards the Rann of Kachchh in Gujarat.

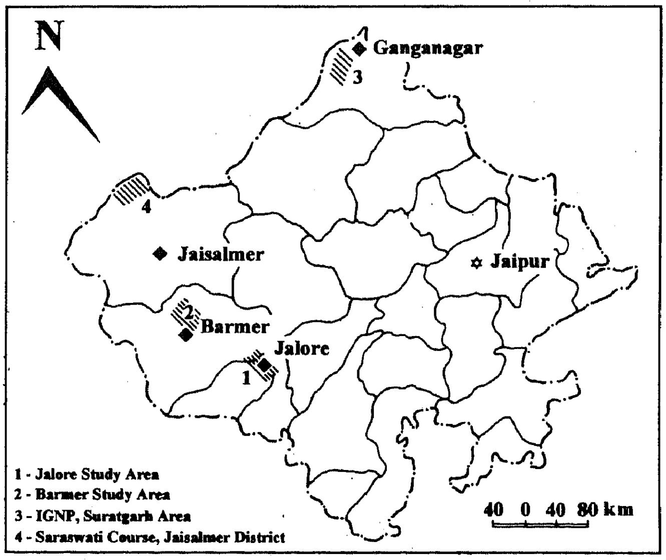

Figure 1. Locations of isotope studies in western Rajasthan.

The younger alluvium is mostly present along the course of Sukri river and comprises unconsolidated to semi-consolidated coarse to fine sand and gravel. The older alluvium of sub-recent to the Pleistocene age consists of semi-consolidated to consolidated coarse to medium sand with clay lenses, caliche and rock fragments. Tertiary sediments, consolidated clays with sand and shale fragments, are encountered at depth in the western as well as southwestern portion of the study area.

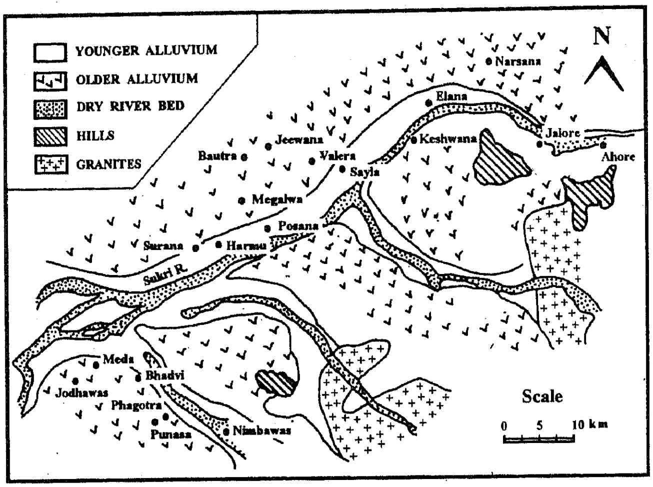

Figure 2. Jalore study area – sample locations (note: Bhadrajun (D14 in Table 1), NNE of Narsana not shown in Figure).

Study of subsurface geology based on borehole data shows that depth to basement increases abruptly west of Sayla probably due to presence of a fault. Sukri river also flows along a NE–SW trending fault. A number of smaller faults are also suspected in the area.

Shallow groundwater mostly occurs under phreatic conditions. Conditions changing from confined to artesian flowing type are observed in the southwestern part. An artesian flowing well is located at Jodhawas which mainly taps the Tertiary aquifer. Groundwater flows towards west-southwest.

A number of samples from shallow (depth < 50 m) and deep (depth > 150 m) wells were drawn from various places (Figure 2) for environmental isotopes as well as major ion chemical analyses[3]. The relevant results are given in Table 1.

In general, fresh, shallow groundwater in the northern part of the study area is of Ca–HCO_3 type. Shallow groundwater along the river course and fresh, deep groundwaters at Punasa and Sayla are of Na–HCO_3 type indicating base exchange. Majority of brackish groundwaters, shallow as well as deep, are of Na–Cl type. Electrical conductivity (EC) of shallow groundwaters ranges from 960 µS/cm to 5300 µS/cm and that of deep groundwaters varies from 1200 µS/cm to 5400 µS/cm.

Table 1. Isotope and other relevant data of samples from Jalore district, Rajasthan

Sample identity	Place	Depth of well (m)	Electrical conductivity (µS/cm)	Chloride (mg/L)	δ^2H (‰)	δ^{18}O (‰)	^{3}H (TU)	^{14}C (pMC)
Deep wells								
T1	Bautra	290	1215	168	−41.8	−5.80	0.8	–
T2	Megalwa	205	3800	801	−51.6	−6.80	0.6	–
T3	Punasa	300	1500	256	−42.2	−6.10	2.4	–
T4	Jodhawas	305	5350	1360	−35.3	−5.10	1.2	12
T5	Meda	280	5400	1333	−35.1	−5.50	1.6	–
T6	Posana	182	3800	781	−50.9	−7.00	0.9	–
T7	Phagotra	150	–	–	−50.2	−6.70	–	–
T8	Sayla	174	1320	229	−42.2	−6.40	3.0	71
Shallow wells								
D1	Bautra	50	1230	162	−39.8	−6.50	1.9	–
D2	Megalwa	12	3950	946	−42.0	−6.60	3.0	–
D3	Punasa	46	1350	162	−44.4	−5.85	4.0	–
D4	Phagotra	40	3550	728	−42.3	−5.65	1.4	–
D5	Narsana	–	3400	751	−35.9	−4.75	5.2	–
D6	Jalore	21	1080	121	−12.5	–	12.0	–
D7	Elana	15	5300	1475	−35.7	−5.25	6.4	–
D8	Jeevana	–	4400	1028	−34.0	−4.25	2.4	–
D9	Harmu	12	2910	362	−46.1	−6.95	1.4	–
D10	Surana	–	1230	149	−39.9	−5.98	17.5	–
D11	Bhadri	–	4000	879	−41.4	−6.70	4.8	–
D12	Nimbawas	41	960	106	−32.5	−4.50	12.1	–
D13	Keshwana	14	3000	624	−30.1	−4.95	11.2	–
D14	Bhadrajun	12	1230	149	−27.5	−3.70	19.5	–
D15	Valera	43	–	–	−53.2	−7.10	–	–
D16	Sayla	15	–	–	−55.4	−7.30	–	–
D17	Posana	–	–	–	−50.4	−7.00	–	–
D18	Ahore	27	1380	199	−42.5	−5.95	2.0	–

Figure 3 shows the plot of δ^2H versus depth of samples from the study area. It could be seen from the plot that the three different aquifers have different isotope signatures. The groundwater in the aquifer situated at about 300 m depth is enriched in stable isotope content compared to that of groundwater occurring at the intermediate depth of about 150–200 m below ground level. In the intermediate aquifer, the groundwater is isotopically most depleted. Most of the samples are brackish. Shallow aquifer shows samples of variable isotope composition with measurable tritium.

Figure 4 shows δ^2H versus δ^{18}O plot of samples from the study area. It could be observed that most of the groundwater samples are depleted in δ^2H and δ^{18}O compared to the 1983 rain events in this area. The samples fall into three distinct categories.

Shallow groundwaters along the river course are isotopically enriched and fall in group C in Figure 4. These samples have higher tritium concentration ranging from 5 TU to 20 TU. These samples fall on a lower slope exhibiting a typical evaporation effect. Shallow groundwater away from the river course and located in the western and southwestern portions of the study area have comparatively depleted stable isotope composition. They fall in group B. Tritium content of these samples ranges from 1.4 TU to 4.8 TU. Some of the shallow wells show depleted stable isotope composition similar to the one exhibited by groundwater in the intermediate aquifer. These samples fall in group A.

In general shallow groundwaters along the river course have enriched stable isotope composition and high tritium. Shallow groundwaters located on the western and southwestern side of the river course and probably along the fault zone have comparatively depleted stable isotope composition and low tritium.

The depleted brackish groundwater (T2, T6, T7) having negligible tritium and occurring at an intermediate depth falls in group A in Figure 4. This aquifer does not appear to be replenished by any recent recharge.

The wells T1, T3 in the deep aquifer and T8 fall in group B in Figure 4. The well T8 taps intermediate aquifer as well as shallow aquifer. This sample shows tritium content of 3 TU and ^{14}C content of 71 pMC (uncorrected age ~ 2800 BP). Samples T1, T3 and T8 are fresh in quality. The stable isotope contents of shallow as well as deep wells (D1, D3 and T1, T3) are similar indicating interconnection between them. Also the deeper aquifer has piezometric surface at an elevation of the shallow water table.

The samples T4 and T5 fall in group C in Figure 4 and are located in the southwestern corner of the area. The well T4 at Jodhawas is of artesian flowing type and the sample has ^{14}C content of 12 pMC (uncorrected age ~ 17500 BP).

It is concluded from the isotope geochemical studies that the shallow aquifer receives recharge by infiltration through river channels during episodic floods caused by sporadic rain events. Some portions of this shallow aquifer also receive recharge from deeper confined aquifers due to upward leakage of groundwater through subsurface fault zones in the area. The deeper (~ 300 m) aquifer was replenished during the cool, pluvial period in early Holocene. The intermediate aquifer (150–200 m) was recharged during the cooler, humid period in the

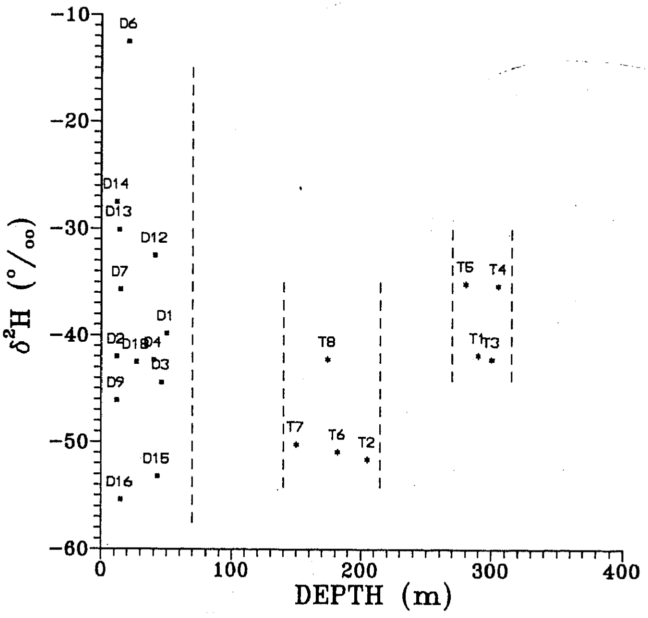

Figure 3. δ^2H vs depth (Jalore samples).

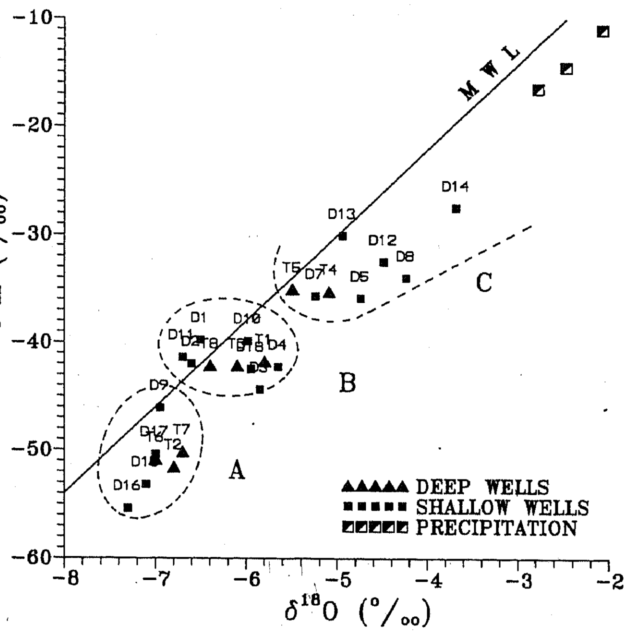

Figure 4. δ^2H vs δ^{18}O (Jalore samples).

mid Holocene. These deeper aquifers do not appear to receive any modern recharge.

Barmer district

An environmental isotope study was undertaken to study groundwater recharge processes in Bhadka–Bheemda area in Barmer district[4]. The study area is arid and receives a mean annual rainfall of 280 mm. A thin veneer of wind blown sand is present over the area. The Tertiary (Eocene) aquifer comprising loosely consolidated to consolidated arenaceous sediment with intercalations of gravel, argillaceous sediment, lignite, etc. and having generally freshwater was of particular interest. Figure 5 shows the sample locations and the geology of the study area. Lathi sandstone of Jurassic age is present on the northern side and Precambrian–Lower Palaeozoic Malani suite of igneous rocks are present on the eastern, southern and western sides. The latter are mainly rhyolites and granites. Two lenticular outcrops of the Tertiary sandstone in the central zone are found to be dry.

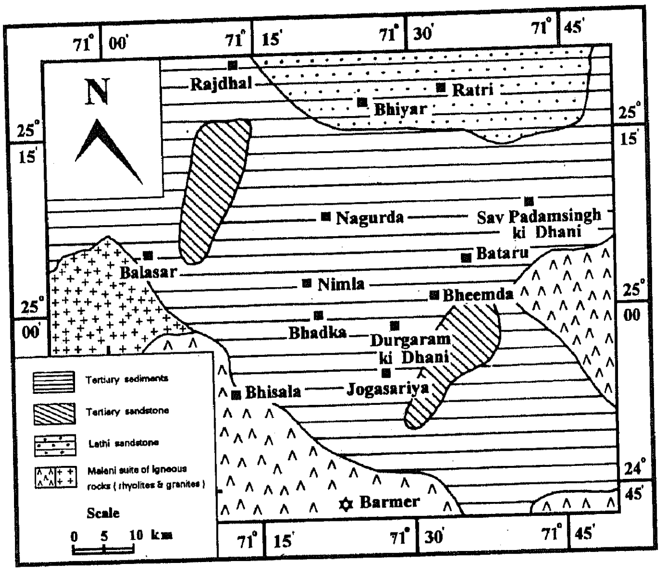

Figure 5. Barmer study area – sample locations.

In the central portion of the area, Nagurda–Nimla–Bheemda, fresh groundwater forms a basin leaving a narrow valley on the eastern and western sides. Shallow aquifers are under phreatic conditions whereas deeper ones change from semi-confined to confined conditions.

Shallow well sample from Balasar is fresh (EC = 630 μS/cm) and is of Ca–HCO_3 type. Other shallow groundwater samples are brackish and of Na–Cl type. The deep fresh groundwater is of Na–HCO_3 type and the deep brackish groundwater is of Na–Cl type. Table 2 lists the isotope and other relevant data of the Bhadka–Bheemda area.

Figure 6 shows δ^2H versus $\delta^{18}O$ plot of samples from the study area. The samples falling in the group A in the plot represent fresh deep groundwater (except the one at Bhadka which is brackish) and most of these are in the groundwater basin in the central portion of the area (except Rajdhal which is situated on the northern side in the dunal area).

The deep and shallow groundwaters from Durgaram ki Dhani and Bataru as well as other shallow samples fall in group B in Figure 6. This group has also the samples from the aquifer in the Lathi sandstone. All these samples show evaporation effect on the stable isotope content. EC also increases along the flow direction in the shallow aquifers. The samples in the group have measurable tritium indicating some component of recent recharge. Higher EC in the shallow aquifer

Table 2. Isotope and other relevant data of groundwater samples from Barmer district, Rajasthan

Sample identity	Place	Depth of well (m)	Electrical conductivity (μS/cm)	δ^2H (‰)	$\delta^{18}O$ (‰)	^3H (TU)	^{14}C (pMC)	^{14}C Model age (BP)
Deep wells								
2	Bheemda	280	1830	– 50.1	– 7.30	0.0	22	7400
3	Jogasariya	285	2450	– 51.7	– 7.70	1.4	25	5500
4	Bhadka	200	4500	– 51.5	– 7.47	0.7	–	–
5	Nimla	220	1560	– 56.1	– 7.98	1.0	50	2400
6	Durgaram ki Dhani	100	3850	– 43.2	– 6.00	0.9	–	–
7	Rajdhal	125	1380	– 53.4	– 8.10	1.7	–	–
9	Bhiyar	100	3000	– 36.3	– 5.20	4.5	–	–
10	Ratri	100	1820	– 35.9	– 5.19	–	–	–
11	Nagurda	95	1710	– 53.4	– 8.00	–	–	–
Shallow wells								
D1	Bheemda	–	6700	– 40.8	– 5.60	6.0	–	–
D2	Bataru	–	5600	– 34.1	– 4.45	3.0	–	–
D3	Durgaram ki Dhani	–	4450	– 45.1	– 6.48	3.0	–	–
D4	Balasar	–	630	– 20.3	– 4.19	21.0	–	–
D5	Sav Padamsingh ki Dhani	70	3200	– 38.9	– 5.03	2.9	–	–
D6	Bhisala	40	4400	– 26.9	– 3.95	–	–	–

is probably the manifestation of the leaching of salts from soils and aeolian sand by water during return flow of irrigation as well as concentration due to evaporation. The samples, shallow as well as deep, from Durgaram ki Dhani and Bataru are a mixture of deep and shallow groundwaters. A shallow sample from Balasar is fresh and contains high (21 TU) tritium. This well probably taps groundwater from weathered igneous rocks. This sample represents water which is about three decades old.

The deeper fresh groundwater is depleted in stable isotopes and has negligible tritium. The model ^{14}C ages[5] for these samples range from 2400 to 7400 BP. This deeper aquifer appears recharged during the cooler, pluvial phase in the Holocene. No modern recharge is discernible. However, if the outcrop of Malani suite of igneous rocks is a probable recharge zone, the groundwater velocity is 6 to 10 m/a.

Indira Gandhi Nahar Pariyojana (IGNP)

The IGNP is a large irrigation and drinking water project to cater to five districts in the western Rajasthan. The Stage I of the canal is in existence for the last 15 years. The canal project is facing twin problems of waterlogging and development of secondary salinity in soils and groundwater in some areas.

The possible causes of waterlogging are: (i) Poor irrigation practices and irrigation return flow, (ii) Non-exploitation of groundwater due to poor quality, (iii) Presence of hydrological barriers at various depths which affect the process of infiltration, (iv) Seepage losses from the canal and its branches.

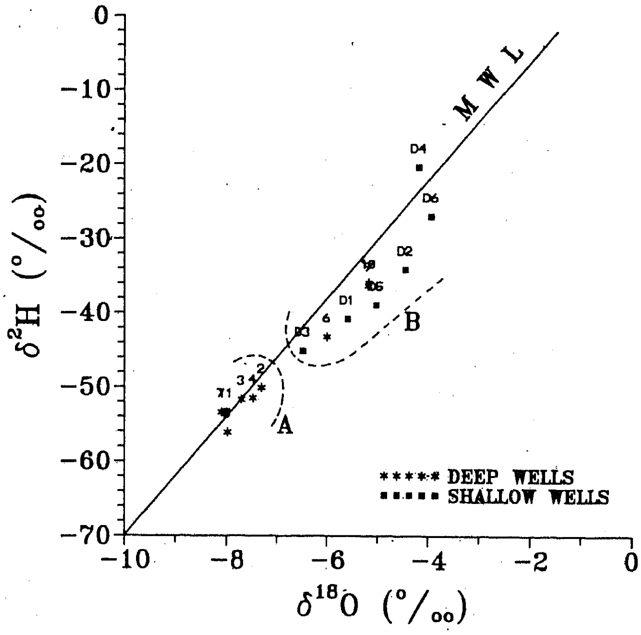

Figure 6. δ^2H vs δ^{18}O (Barmer samples).

Isotope investigation has been initiated in Suratgarh area (Figure 1) in collaboration with the Groundwater Department, Rajasthan to study the contribution to groundwater from canal seepage and irrigation return flow as well as interconnections between shallow and deep aquifers.

Figure 7 shows δ^2H versus δ^{18}O plot of samples collected in the ongoing study. The preliminary observations indicate that the canal water originating from the higher altitudes in the north is depleted in stable isotope composition compared to local precipitation and groundwater. These samples fall in group A. Groundwater samples influenced by seepages from the canal fall in group B. These samples fall on a regression line with equation, $\delta^2H = 8.2\delta^{18}O + 17.3$ ($r = 0.975$; $n = 12$). Piezometer samples affected by irrigation return flow, waterlogging and saline groundwaters fall in the group C showing evaporation effect.

Tritium level of canal water (~ 12 TU) is slightly higher than that of the precipitation (~ 9 TU). Native saline groundwaters show negligible tritium.

The canal seepage to groundwater consists of two end points; canal water and pure groundwater and the admixture. If the stable isotope compositions of the end points and those of the admixtures are known, it is possible to calculate the percentage contribution of canal water to the admixture. It is estimated that the contribution is about from 3 to 5% for a period of 15 years or so.

Lost courses of Saraswati River

The legendary 'Saraswati' river has been described as a mighty Himalayan river in the ancient Indian literature,

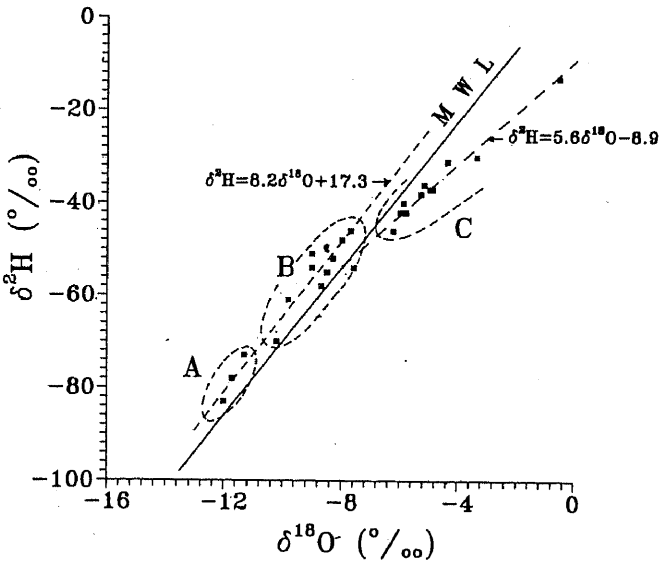

Figure 7. δ^2H vs δ^{18}O (IGNP samples).

e.g. *Rigveda*[6]. Thereafter, there have been many references to the Saraswati river and its disappearance. Early literature indicates the existence of the river before 3000 BP. Most of this literature was, however, aimed at studying the relics of the once great Indus Valley Culture in the region. Many attempts have been made to explore the former courses of the Saraswati river in the last and this century. Allchin *et al.*[7] have summarized these findings in the following way:

> 'Evidence from many sources, including that of archaeological remains associated with older river courses, indicates that a major river, stemming mainly from the same sources as the present Sutlej, flowed through Northern Rajasthan, Bahawalpur and Sind – to the southeast of the present course of the Sutlej and the Indus – in the third to second millennium BC. This river known as Saraswati in its upper course, at different times either joined the lower course of the Indus in Sind, or found its way independently into the Arabian sea via Rann of Kutch' (p. 198).

The river is supposed to have changed its course several times in the westward direction. Recently, interpretation of LANDSAT imagery of the western part of Jaisalmer district revealed buried courses of the river running from NE to SW[8,9]. This course is found to have links with the dry bed of Ghaggar river in the northeast (Ganganagar district) while in the southwest it met with or even cut across the surviving courses of the Hakra and Nara rivers in Pakistan.

In northwestern part of Jaisalmer district, in spite of very low rainfall (less than 150 mm) and extreme conditions of the desert, groundwater is available at depth of about 50–60 m along the course of the defunct river and a few dug wells do not dry up throughout the year. It is found that the area through which the river bed is traced supports vegetation even during summer. It is thought that these courses of river in the area still maintain their head water connection and could form potential groundwater sanctuaries for exploitation.

To confirm the scenario, an environmental isotope study was undertaken in collaboration with the Groundwater Department, Rajasthan in the Jaisalmer district. A set of samples collected in 1995 was analysed for isotopes δ^2H, δ^{18}O, δ^{13}C ^{3}H and ^{14}C. The preliminary observations are mentioned below.

The groundwater in the area is enriched in stable isotope content (δ^{18}O: -6 to -2‰) compared to that of Himalayan rivers (δ^{18}O: -11 to -10‰). The groundwater samples exhibit negligible tritium content indicating absence of modern recharge. Radiocarbon data suggest the groundwater is a few thousand years old. The ^{14}C levels decrease along the suspected river course downstream (58.8 pMC to 54.9 pMC) indicating hydraulic continuity of the Saraswati buried channel from Kuria Beri to Ghantiyalji. A groundwater velocity of about 20 m/a is estimated from ^{14}C data. Further work is in progress.

Concluding discussion

Deserts occupy waste regions mainly in the latitude belts between 15° and 30°. The Sahara desert in the Northern Africa, the largest in the world has a surface area of about 9 million km^2. Other large deserts are those of Arabian Peninsula and Iran (which might be considered a continuation of the Sahara), the Turkesthan desert, the Thar desert in India, the Gobi desert in Mongolia, the Great American desert and in southern hemisphere the Kalahari desert in South Africa, the Great Australian desert and Atacama desert in Northern Chile. The last, though relatively small in size, is the driest – no precipitation has been recorded over most of it for more than one century.

Western Rajasthan, which forms part of the Thar desert, has all the attributes of an arid zone – aeolian topography concealing geological features and formations, scarce and erratic rainfall and large evapotranspiration. Evidence from archaeological sites, radiocarbon and thermoluminescence dating of quartz sediment and pot shreds show that the dunes were accumulating from at least 20000 BP and were stable by the middle to late Holocene. The continuation of dune construction into the Holocene distinguishes the Thar from other mid-latitude dunefields[10].

The stabilization of dunes in Africa and Australia, and probably Arabia, broadly coincides with the global warming that post-dated the Last Glacial Maximum (LGM)[11,12]. The monsoon circulation ceased to exist during LGM[13]. It is most likely that the monsoon circulation re-established during the global warming that began around 14000 BP (ref. 11). This warming peaked in the period 8000–6000 BP, along with a general water surplus which, in many places, has been interpreted as the result of increased rainfall[14].

The history of lacustrine sedimentation in the Thar shows that hypersaline conditions prevailed at about the LGM and violent fluctuations of water level occurred between this maximum of aridity and the onset of freshwater conditions about 6000 BP. Freshwater, high lake level conditions prevailed until about 4000 BP when sediments rather like those of today began to be deposited[10].

Singh *et al.*[15] carried out a pollen analytical study of lakes, providing a Holocene record of vegetation changes in the northern Thar. They were able to propose a series of climatic changes:

Phase 6	1100–0 BP	Conditions same as today (ephemeral playas with very thin halite crust)
Phase 5	3000–1100 BP	Drying of lakes and lack of pollen
Phase 4	5000–3000 BP	Wettest period – lacustrine conditions and swamp vegetation, increased rainfall
Phases 2 and 3	10000–6000 BP	Wet period – lacustrine conditions with rainfall greater than at present
Phase 1	Beyond 10000 BP	Active dune development

Isotope hydrological studies carried out in western Rajasthan essentially echo the climatic changes mentioned above, especially phases 2, 3 and 4. The replenishment of groundwater resources took place in the early to mid Holocene in the Thar desert. The depleted stable isotopic composition and low radiocarbon content of groundwaters in deeper aquifers suggest cool, pluvial phase(s) in the Holocene responsible for the replenishment. Moreover, comparison of isotopic composition (δ^2H and $\delta^{18}O$) and radiocarbon content of groundwaters from Jalore and Barmer areas indicates that the climatic conditions in the Thar desert changed from cool, pluvial in early Holocene to cooler, pluvial in the mid-Holocene. However, more comprehensive isotope hydrological data will shed more light on the climatic aspects of the Thar desert.

It is true that groundwater is relatively abundant under most of the deserts, but is seldom easily accessible. Also practical implementation of rational exploitation of groundwater and agricultural development of desert is rather problematic. In most of the deserts, including the Thar desert, the need for an accurate assessment of whether a small quantity of rainfall recharge is occurring appears rather academic when the withdrawal from an aquifer clearly surpasses the present day maximum possibilities of infiltration through catchment area. The heavy withdrawal of groundwater will not only pose the problems of accessibility but also of the stability of the ground due to pressure decrease as well. The conjunctive use of precious groundwater resources with improved irrigation practices will help arrest the secondary soil and groundwater salinization.

Isotope techniques are an important tool in arid-zone hydrology. When applied effectively with multi-disciplinary approach, one can evaluate groundwater recharge, investigate recharge processes, understand the dynamics of groundwater, study mechanism of salinization and related problems among others. The information will also help in planning the development of groundwater resources through artificial recharge of aquifers. From the foregoing, it is clear that isotope techniques should form an integral part of any hydrogeological investigation undertaken for the development of groundwater resources, particularly in the arid zones.

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Solar energy in western Rajasthan

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This article reviews the state-of-the-art for generating electricity from solar energy, through the photovoltaic and thermal routes. On the whole, India has made significant efforts for the successful utilization of solar energy. A large number of kilowatt scale PV power packs and systems for a variety of applications have been installed. Successful attempts have also been made to integrate a few PV systems with the grid. As regards the thermal route, the experience of generating electricity has been limited. However, ambitious plans are afoot for setting up a few large power plants in western Rajasthan where the conditions are most appropriate for the utilization of solar energy.

ENERGY from the sun sustains life on our earth. It is also the source of almost all forms of energy used by man. Fossil fuels such as coal, oil and natural gas represent solar energy that was received on earth millions of years ago, while renewable sources of energy like wind, hydroelectric power, biomass and ocean energy are indirect forms of solar energy.

Solar energy, as received directly, can be used in a number of ways and for many applications. The two routes for utilization are the thermal route using the heat for heating, cooling, drying, water purification, power generation, etc. and the photovoltaic route which converts the light into electricity which can then be used for a variety of purposes such as lighting, pumping, communication and refrigeration. In this article, we focus on various technologies and systems using solar energy directly for generating electricity.

India receives solar energy equivalent to over 5000 trillion kWh/year which is many times more than the total energy consumption of the country. The daily average incident solar energy varies from 4 to 7 kWh/m^2 depending upon the location and there are 250–300 sunny days in most parts of the country. The potential for using solar energy to meet the growing energy needs of the country was recognized in the mid seventies. Research and development efforts in both solar thermal and photovoltaic routes were initiated. Several applications were developed and demonstrated during the eighties. These efforts received an impetus with the establishment of the Ministry of Non-Conventional Energy Sources (MNES) in 1992. The Ministry's programmes include R&D, demonstration, commercialization and utilization activities in respect of a wide variety of renewable energy technologies. The programmes are implemented through a network of state level agencies, industrial, research and academic organizations and non-governmental bodies. As a result of these efforts, India today has one of the world's largest programmes in solar energy. Solar energy is beginning to be used for a large number of applications. Nevertheless, the achievements so far add up to only a tiny fraction of what is possible. The efforts initiated during the last three years by MNES to restructure the programmes and give them a market orientation could lead to a significant acceleration in the utilization of solar energy in the country.

Availability of solar radiation over western Rajasthan

Rajasthan is the second largest state in size in the country, covering about 10.4% of the land area. The state has limited traditional sources of energy. It has only two perennial rivers, the Chambal and the Mahi, whose hydroelectric potential has been almost fully exploited. There are no coal mines or oil fields. However, there are some gas and lignite reserves.

In order to discuss the potential for using solar energy in western Rajasthan, it is desirable to examine the climatic conditions of that region. The variables of importance from the point of view of generating solar electricity, are solar radiation, sunshine hours, clearness index and ambient temperature.

Western Rajasthan experiences a hot and dry climate with a severe summer, relatively clear skies, a short monsoon period and a cold winter. Table 1 presents annual and monthly averages of daily values of global solar radiation, sunshine hours and clearness index for four towns of the region. In order to get a comprehensive picture of the spatial distribution of these quantities over the whole area, a map showing the distribution of the annual mean daily global solar radiation and the clearness index is presented in Figure 1. It is seen that the solar insolation level is high. In fact, the values over western Rajasthan are the highest for the whole country. Further, the sunshine hours and values of clearness index for the region are consistently high, except for a short monsoon period. This implies an excellent availability of beam radiation. Consequently solar devices which need concentration of radiation would perform much better in this region. Besides these factors, the ambient temperatures are high throughout the year touching more

Table 1. Global solar radiation (G in kWh/m^2 day), sunshine hours (S) and clearness index (K_T) in western Rajasthan[4,5]

Place	Parameter	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.
Barmer	G	4.69	5.62	6.57	7.47	8.06	7.89	6.55	6.26	6.62	6.07	5.07	4.47
	S	8.9	9.6	10.1	11.0	12.0	11.2	7.3	7.1	9.5	10.2	9.5	8.8
	K_T	0.72	0.72	0.72	0.72	0.73	0.70	0.59	0.59	0.69	0.73	0.73	0.72
Bikaner	G	4.46	5.37	6.38	7.49	8.12	8.15	7.29	6.83	6.79	5.96	4.80	4.21
	S	8.8	9.4	10.0	11.3	12.2	12.0	9.6	8.9	10.4	10.4	9.3	8.7
	K_T	0.72	0.71	0.71	0.73	0.74	0.72	0.65	0.65	0.71	0.74	0.73	0.72
Jaisalmer	G	4.61	5.56	6.49	7.48	8.11	8.24	7.44	7.08	6.81	6.06	5.00	4.36
	S	9.0	9.7	10.1	11.2	12.2	12.3	10.0	9.6	10.3	10.5	9.6	8.8
	K_T	0.72	0.73	0.71	0.73	0.74	0.73	0.67	0.67	0.71	0.74	0.74	0.72
Jodhpur	G	4.72	5.57	6.55	7.23	7.55	7.07	5.98	5.54	6.10	5.83	4.90	4.43
	S	9.1	9.7	8.9	10.0	10.6	9.5	6.7	6.2	7.7	9.5	9.7	9.2
	K_T	0.72	0.72	0.68	0.69	0.68	0.64	0.56	0.57	0.65	0.72	0.74	0.73

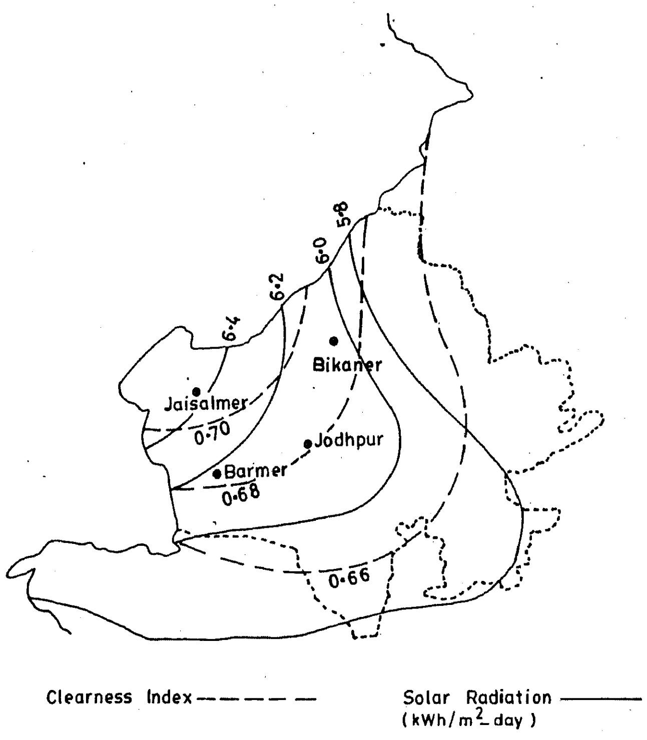

Figure 1. Distribution of annual mean daily global solar radiation and clearness index over western Rajasthan[5].

than 45°C during the summer. Thus the region is the best in the country from the point of view of utilizing solar energy.

Photovoltaic technology

Photovoltaic (PV) technology enables the direct conversion of sunlight into electricity. A photovoltaic module is the basic building block of a PV system. The power from the PV module can be used to run pumps, lights, refrigerators, TV sets, etc. The PV modules, batteries, charge controller, inverter (if any), mounting structure, cables, switches and the appliances being powered together constitute a complete PV system.

PV power systems have several advantages. They are modular in nature and have no moving parts, produce no noise or pollution and require very little maintenance. They can be installed almost anywhere, making them an ideal power source for use in remote and isolated areas which are not served by conventional electricity.

With 75,000 villages still to be electrified, there is a vast scope and potential for use of PV technology in India. Recognizing this fact, the government has been implementing a comprehensive programme covering R&D, demonstration, commercialization and utilization aspects for over 15 years. The technology for the fabrication of solar cells using single crystal silicon wafers has been developed and commercialized in the country based on indigenous efforts. This was followed by further demonstration of the applications in different sectors and the development of newer technologies such as amorphous silicon and other thin film technologies.

India has acquired a leading status among the countries of the world in the development and use of PV technology. The Indian market and programme are the largest among developing countries and India has emerged as the second largest manufacturer of PV modules based on crystalline silicon technology. Industrial production touched a level of 7 MW during 1995–96. There are over 75 companies now engaged in the production of solar cells, modules and systems. There are seven solar cell manufacturers and ten PV module manufacturers in production.

Some applications for which PV systems have been developed are:

1. Pumping water for irrigation and drinking.
2. Electrification for villages for providing street lighting and home lighting and for providing community services.

3. Telecommunication for the posts and telegraph and railway communication network, and for other specialized purposes.

A diagram of a typical system for pumping water from a bore well is shown in Figure 2. A solar cell array supplies power through a dc–ac inverter to an electric motor coupled to a submersible pump. The pump is installed below the water level of the bore well. Its discharge is connected through a delivery pipe to a storage tank at ground level. Water can be withdrawn from the storage tank for use when required. Usually systems for pumping water do not have storage batteries and work only during the day when adequate solar radiation is available. However, other PV systems which have to supply power during the evening or night have storage batteries to store the electrical energy generated during the day, the capacity of the battery being determined by the nature of the application.

Rajasthan is one of the leading states in the country in utilizing solar photovoltaic technology for decentralized and stand-alone systems. With assistance from the Central Government, the Rajasthan Energy Development Agency (REDA) has been implementing a programme for providing PV lighting systems in its rural and remote villages. Under this scheme the State has deployed 5545 street lighting systems, 4700 solar lanterns and 115 community TV systems.

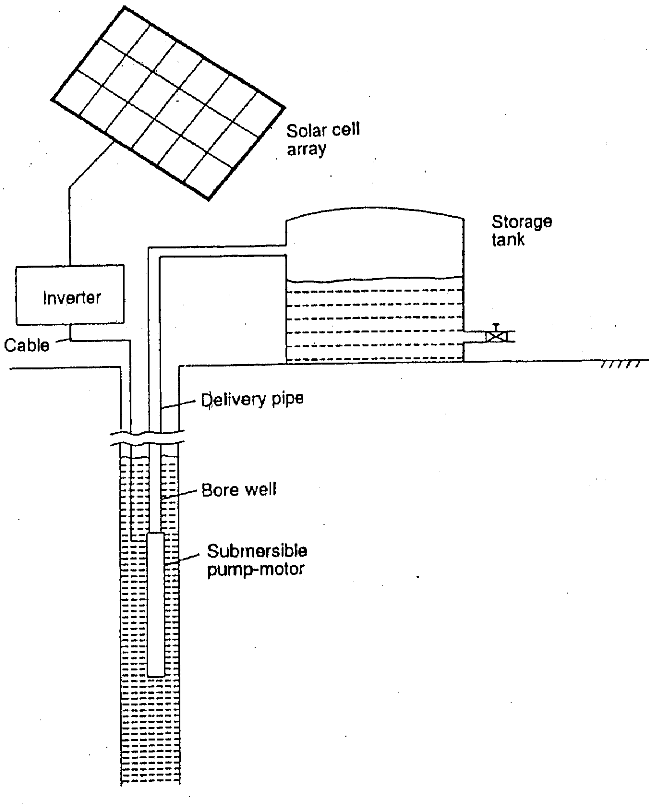

Figure 2. A photovoltaic water-pumping system.

Nineteen village level PV power packs of 113 kWp aggregate capacity have also been installed till June 1996 (ref. 1). The PV power packs comprise individual home lighting systems for each household, a few street lighting systems, a community TV system for education and entertainment and a community drinking water pumping system. A typical power pack has a capacity of about 10 kW peak and comprises 80 home lighting systems, 20 street lighting systems, two community TV systems and a community water pumping system. Such systems have been functioning satisfactorily for the last 5 years.

Most of these power packs have been installed in villages in Jaisalmer and Udaipur districts. Over the years, REDA has evolved a method for maintenance and servicing of these power packs with the active co-operation of the beneficiaries and system suppliers.

The Rajasthan Government has also proposed that 552 villages in the districts of Udaipur, Jaisalmer and Ganganagar be electrified with PV power packs. The above villages are not likely to be electrified by conventional grid power during the next 5–10 years. The aggregate PV capacity of these systems is about 1.44 MWp and the total cost is likely to be about Rs 66 crores.

Recently efforts are also being directed towards establishing and demonstrating the potential of solar photovoltaics to generate larger amounts of power which would supplement and augment grid power. MNES has completed two pilot village electrification projects of 100 kW each, with 25 kW grid connected components. The projects have provided initial insights for the design of high voltage systems, power conditioning unit, grid integration, etc. The Ministry is now in a position to demonstrate various grid-connected possibilities based on new design concepts and more such projects are planned. It is expected that higher conversion efficiencies and optimally engineered systems will bring about rapid market expansion, which will eventually lead to a decline of costs of PV systems. This stage is likely to be reached by the turn of the century when solar photovoltaics could approach competitiveness for grid-connected applications.

Keeping in mind the above developments, the Government of Rajasthan has issued Letters of Intent for the establishment of two large-sized PV solar power plants which would be connected to the grid. Both these plants have a capacity of 50 MW. One is based on PV thin film technology and the other on PV modules using concentrators. The plants are to be established on a build, own, operate and maintain (BOOM) basis and the commitment of the State Government is to buy the power generated from these plants at the following rates:

Rs 2.25 per unit for the first three years,
Rs 2.70 per unit for the fourth year,
Rs 3.82 per unit for the next seven years and

Rs 2.25 per unit for the remaining period up to 25 years.

Solar thermal power generation systems*

Solar thermal power systems can be classified as working on low, medium and high temperature cycles. Low-temperature cycles work at maximum temperatures of about 100°C, medium-temperature cycles work at maximum temperatures up to 400°C, while high-temperature cycles work at temperatures above 400°C (ref. 2).

Low-temperature systems use flat-plate collectors or solar ponds for collecting solar energy. Recently, systems working on the solar chimney concept have been suggested. Medium-temperature systems use the line focussing parabolic collector technology. High-temperature systems use either paraboloidal dish collectors or central receivers located at the top of towers.

Low-temperature systems

A diagram of a typical low-temperature system using flat-plate collectors and working on a Rankine cycle is shown in Figure 3. The energy of the sun is collected by water flowing through the array of flat-plate collectors. The hot water at temperatures close to 100°C is stored in a well-insulated thermal storage tank. From here, it flows through a vapour generator through which the working fluid of the Rankine cycle is also passed. The working fluid has a low boiling point. Consequently, vapour at about 90°C and a pressure of a few atmospheres leaves the vapour generator. This vapour then executes a regular Rankine cycle by flowing through a prime mover, a condenser and a liquid pump. The working fluids normally used are organic fluids like methyl chloride and toluene, and refrigerants like R-11, R-113 and R-114.

It has to be noted that the overall efficiency of this system is rather low, because the temperature difference between the vapour leaving the generator and the condensed liquid leaving condenser is small. For the cycle shown in Figure 3, the Rankine cycle efficiency is about 7–8%. The efficiency of the collector system is of the order of 25%. Hence an overall efficiency of only about 2% is obtained. Because of the low overall efficiency, large collector areas are required and such plants have been found to be very costly. Typically the installed cost is about Rs 300,000 per kW for 6 to 8 h of daily operation, the main component of cost being the collectors.

In order to reduce the cost, solar ponds have been used instead of flat-plate collectors. The first two solar pond power plants having capacities of 6 kWe and 150 kWe were constructed in Israel about 15 years ago. These were followed in 1984 by the Bet Ha-Arava power plant, the largest in the world with a capacity of 5 MW. The working of these plants has established the technical viability of solar pond power plants. However, they also do not appear to be economically attractive.

Recently the concept of a solar chimney power plant has been suggested. In such a plant, a tall central chimney is surrounded at its base by a circular greenhouse consisting of a transparent cover supported a few metres above the ground by a metal frame (Figure 4). Sunlight passing through the transparent cover causes the air

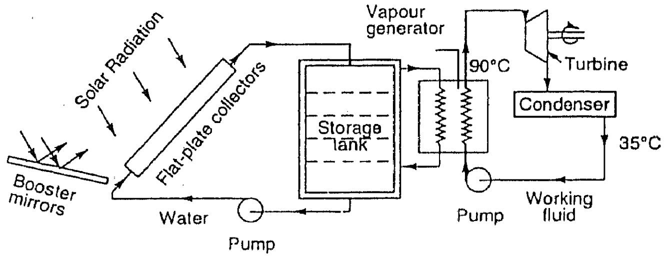

Figure 3. Low temperature power generation cycle using flat-plate collectors.

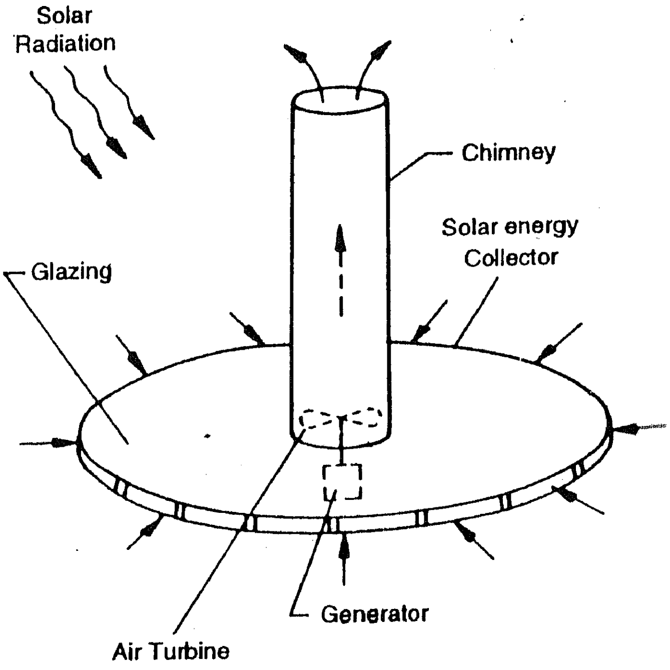

Figure 4. Solar chimney power plant.

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*From a historical view point, it is of interest to note that the first experiments on the generation of solar thermal power in India were conducted by an Englishman, William Adams, more than one hundred years ago. Adams stayed in Mumbai and performed his experiments in the compound of his bungalow. He has described his work in a book entitled *Solar Heat – A Substitute for Fuel in Tropical Countries for Heating Steam Boilers and Other Purposes* (Education Society's Press, Byculla, Mumbai, 1878).

trapped in the greenhouse to heat up. A convection system is set up in which this air is drawn up through the central chimney turning a turbine located near the base of the chimney. The hot air is continuously replenished by fresh air drawn in at the periphery of the greenhouse.

The only solar chimney power plant built so far is a 50 kW pilot plant in Spain. It has a 200 m high chimney with a diameter of 10.3 m. The solar collector area extends to a radius of 126 m from the chimney with the glazing being 2 m above the ground. The turbine, housed at the base of the chimney, has four 5 m long blades and rotates at 1500 rpm to produce an output of 50 kW.

Although the energy conversion efficiency of such plants is low (less than 1%), it is claimed that there will be considerable reduction in cost with scale-up and that a large size 1000 MW plant may cost only about $ 1000 per kW.

Medium-temperature systems

Among solar thermal-electric power plants, those operating on medium-temperature cycles and using the line focussing parabolic collector technology at a temperature of about 400°C have proved to be the most cost effective and successful so far. A schematic diagram of a typical plant is shown in Figure 5. The first commercial plant of this type having a capacity of 14 MW was set up in 1984. Since then six plants of 30 MW capacity each, followed by two plants of 80 MW each have been installed and commissioned. All these plants have been set up in California, which has a total installed capacity of 354 MW. The collector array for the 80 MW plant has an area of 464,340 m^2. The cylindrical parabolic collectors used have their axes oriented north-south. The absorber tube used is made of steel and has a specially developed selective surface. It is surrounded by a glass cover with a vacuum. The collectors heat a synthetic oil to a temperature of 400°C with a collector efficiency of about 0.7 for beam radiation. The synthetic oil is used for generating superheated high pressure steam which executes a Rankine Cycle with an efficiency of 38%. The plant generally produces electricity for about 8 h a day and is coupled with natural gas for continuous operation. The installed cost of this type of plant has reduced over the years because of the increasing installed capacity. The latest 80 MW plant is reported to have cost $ 2900 per kW. The generating cost is about 8 cents per kWh.

The Indian experience with the line focussing parabolic collector technology has been restricted so far to a small

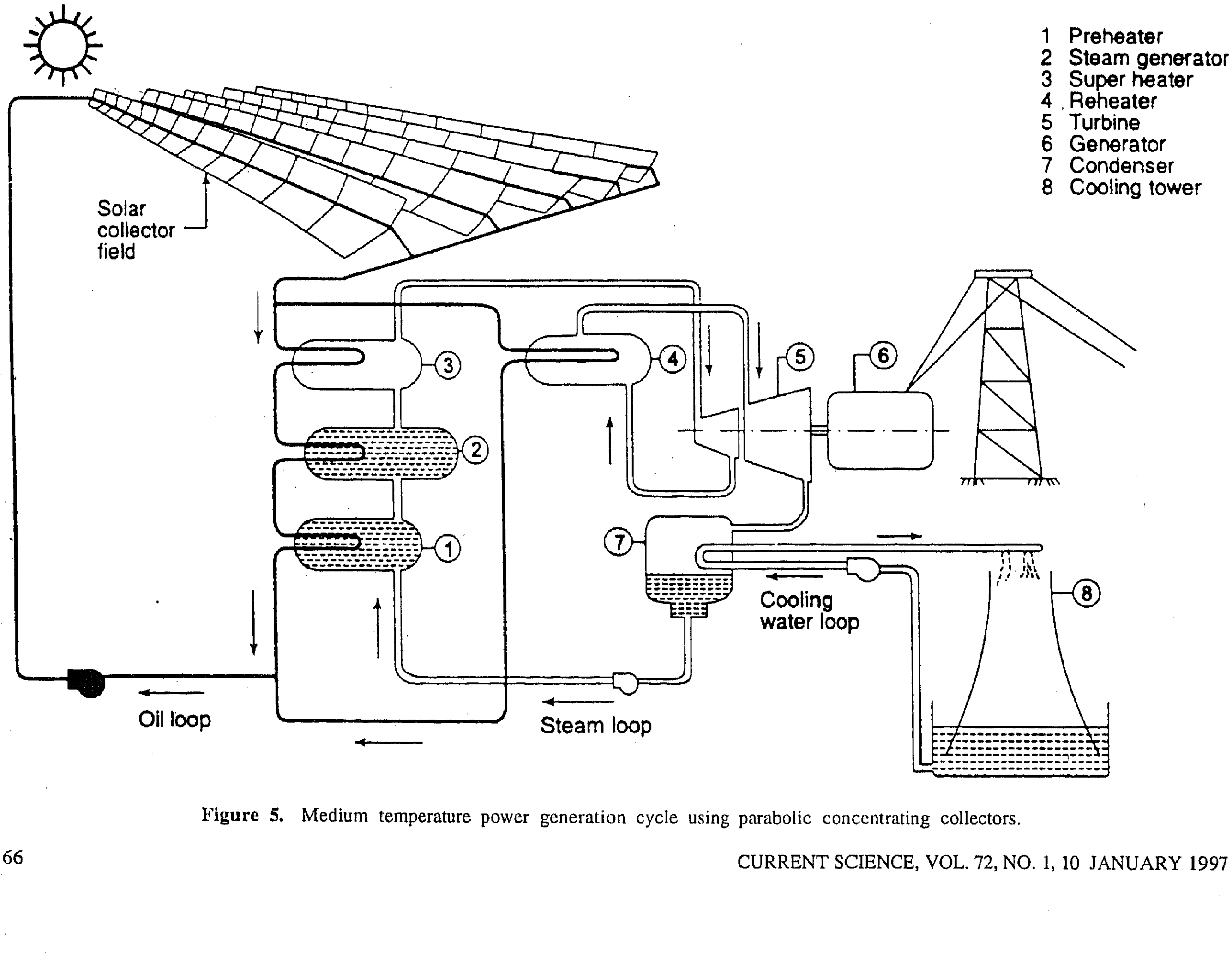

Figure 5. Medium temperature power generation cycle using parabolic concentrating collectors.

50 kW capacity experimental plant installed at the Solar Energy Centre near Delhi.

High-temperature systems

Two concepts have been tried with high-temperature systems. These are the paraboloidal dish concept and the central receiver concept.

In the paraboloidal dish concept, the concentrator tracks the sun by rotating about two axes and the sun's rays are brought to a point focus (Figure 6). A fluid flowing through a receiver at the focus is heated and this heat is used to drive a prime mover. Typically Stirling engines have been favoured as the prime movers and systems having efficiencies up to 30% and generating power in the range of 8 to 50 kWe have been developed.

The Indian experience with this type of system has been restricted to a small 20 kW power station near Hyderabad set up in the eighties. Four paraboloidal dish collector modules were used to generate steam which ran a steam engine.

Because of limitations on the size of concentrator, paraboloidal dish systems would generally be expected to generate power in kilowatts rather than megawatts. Thus they can only be expected to meet the local power needs of communities, particularly in rural areas.

In central receiver power plants, solar radiation reflected from arrays of large mirrors (called heliostats) is concentrated on a receiver situated at the top of a supporting tower. A fluid flowing through the receiver absorbs the concentrated radiation and transports it to the ground where it is used to operate a Rankine power cycle. A schematic diagram showing the main components of a central receiver power plant in which water is converted into steam in the receiver itself is shown in Figure 7. Alternatively the receiver is used to heat a liquid metal or a molten salt and this fluid is passed through a heat exchanger in which steam for the power cycle is generated. Seven central receiver power plants have been built in the eighties. All these plants were pilot plants and had outputs ranging from 0.5 to 10 MWe. A few details are given in Table 2. These include the number and the size of the heliostats, the receiver type, the receiver fluid and the height of the central supporting tower.

Although all the central receiver plants have been operated successfully, the available data indicate that the construction cost was very high. For example, the largest plant, Solar One, at Barstow, California cost approximately $ 14,000 per kW. However, costs are likely to reduce with more operational experience, improved design and scale-up.

Recently, Solar One has been converted from a water–steam system to a more advanced system using a molten salt. The molten salt at 290°C is pumped from a cold storage tank through the receiver where it is heated to 560°C, and then on to a hot storage tank. When electricity is to be generated, the hot salt is pumped to a steam-generating system that produces superheated steam for the turbine/generator. After giving up its heat, the salt

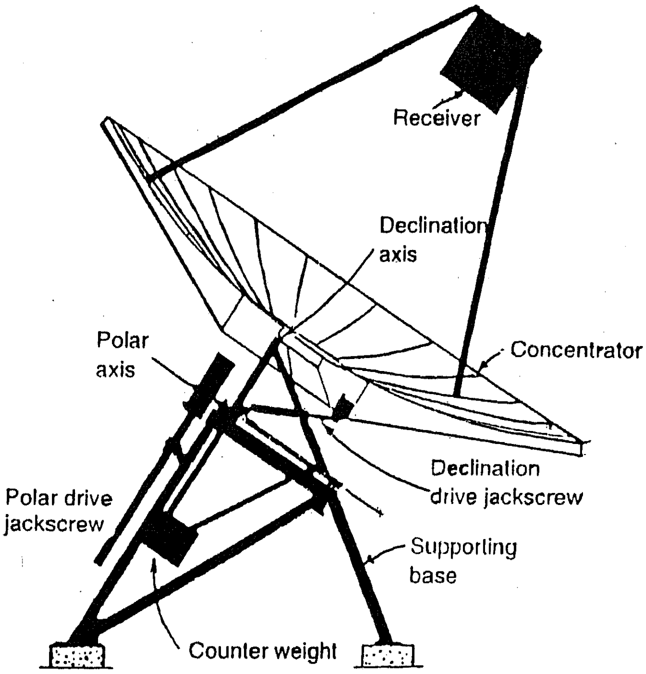

Figure 6. Paraboloid concentrating collector.

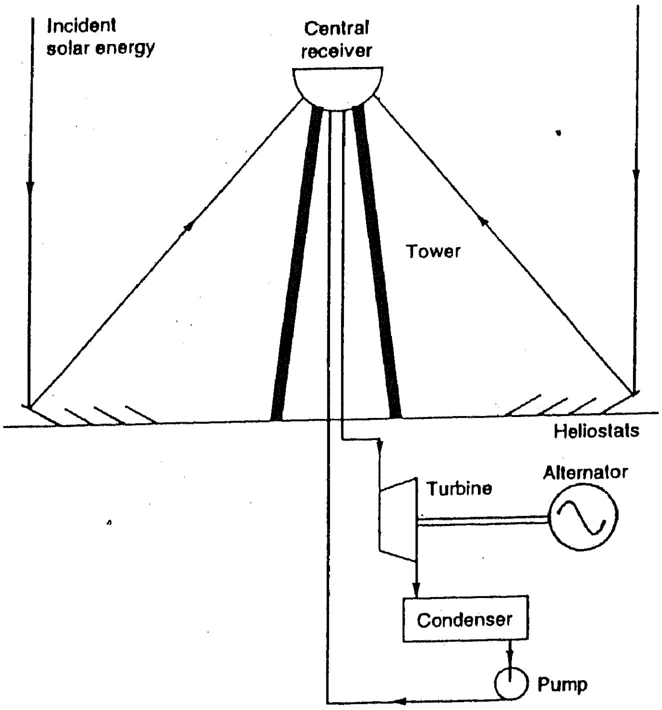

Figure 7. Central receiver power plant.

Table 2. Solar central receiver power plants[2]

Plant name	SSPS	Eurelios	CESA I	Sunshine	Themis	CES 5	Solar One
Location	Spain	Italy	Spain	Japan	France	USSR	USA
Output (MWe)	0.5	1	1.2	1	2	5	10
Number of heliostats	93	112,70	300	807	201	1600	1818
Area of heliostat (m^2)	39.3	23,52	39.6	16	53.7	25	39.3
Total reflecting area (m^2)	3655	6216	11880	12912	10740	40000	71447
Receiver type	Cavity	Cavity	Cavity	Cavity	Cavity	External	External
Receiver fluid	Sodium	Steam	Steam	Steam	Molten salt	Steam	Steam
Tower height (m)	43	55	60	69	–	70	80
Start of operation	1981	1981	1983	1981	1983	1985	1982

is returned to the cold storage tank. The converted plant, called Solar Two, began operation in June 1996 (ref. 3). It is expected that the use of molten salt will result in a higher efficiency and a better operating performance.

The Government of Rajasthan has also issued a Letter of Intent for a large 200 MW solar thermal power plant. The plant would be established on a BOOM basis and the State Government has made a commitment to purchase power at the same rates as for the two proposed 50 MW PV-based plants. The indications given are that the plant would be based on the solar chimney concept and located in Jaisalmer district. The plans are to construct the plant in stages with the chimney height as well as the collector area on the ground being increased in steps. The chimney would initially be 300 m in height, then raised to 600 m and finally to a height of 1000 m. The total collector area would finally be about 100 million m^2.

The other large power plant planned for Rajasthan is proposed to be located in Jodhpur district. It will be a 140 MW integrated solar combined cycle power plant with about 35 MW being generated from solar energy using the line focussing parabolic collector technology. The balance power of about 105 MW will be generated by conventional means using fossil fuels[1]. It is expected that this mix would be the most techno-economically feasible and viable option. A detailed feasibility report has been prepared and a laboratory established for collection of meteorological data at the selected site. Indications are that the total project cost may be of the order of $ 280 million. The mode of implementation, pattern of funding, and the role of various organizations are being finalized.

Concluding remarks

We have reviewed the state-of-the-art for generating solar electricity by the photovoltaic as well as the thermal route. As far as PV-based systems are concerned, the country has done well in developing indigenous technology and in setting up a large number of kilowatt scale power packs and systems for a variety of applications. Successful attempts have also been made to integrate these systems with the grid on the kilowatt scale. In so far as the thermal route is concerned, the domestic experience has been limited. However, an ambitious 140 MW integrated plant is planned for western Rajasthan. This should give the right impetus to the solar power generation programme.

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Correction in the reported amino acid sequence for human seminal plasma prostatic inhibin

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NMR studies on human seminal plasma prostatic inhibin (HSPI) reveal that the amino acid at position 93 is Ile and not Gly as was reported earlier. This suggests that HSPI and prostatic secretory protein are one and the same protein.

HUMAN seminal plasma contains a variety of proteins secreted by seminal vesicles and the prostate. Human seminal plasma prostatic inhibin (HSPI), prostatic secretory protein (PSP-94), immunoglobulin binding factor (IgBF) and β-microseminoprotein (β-MSP) are among the human seminal proteins which have been isolated, purified and characterized during the last decade. All these proteins have close (~ 99%) sequence homology. HSPI, first isolated by Sheth and his coworkers[1], has biological activity ranging from preventing pregnancy to curing prostate cancer. Inhibin prevents pregnancy in mammals by modulating the level of circulating follicle stimulating hormones (FSH)[2]. It suppresses prolactin, a hormone that promotes lactation[3]. Therefore, neutralizing inhibin through active immunization leads to increase in milk production. Seidah *et al.* have reported the primary sequence of HSPI[4]. This was followed by the discovery of PSP-94 (ref. 5) which showed complete sequence homology with that of HSPI except for one amino acid residue at position 93. HSPI is reported to contain Gly at this site whereas PSP-94 has Ile (Figure 1).

Around the same time, Kazuko *et al.* isolated β-MSP[6] which was reported to be a 93 residue protein showing differences in sequence homology at positions 39 and 40 from PSP-94. While β-MSP contains Pro and Thr at these positions, PSP-94 and HSPI have Thr and Cys, respectively (Figure 1). Another group, Zhi *et al.*[7] who purified and characterized IgBF, reported the primary sequence of only thirty N-terminus and two C-terminal amino acids of IgBF, which show complete sequence homology with the corresponding stretches of PSP-94. On the basis of such sequence homologies, Zhi *et al.*[7] suggested that HSPI, PSP-94, β-MSP and IgBF may be the same protein.

No information is available about the three-dimensional (3D) structure of these proteins. We have undertaken studies on the 3D structure of HSPI using multidimensional NMR spectroscopy. Our investigations unequivocally establish that the amino acid at position 93 is Ile and not Gly.

Semen samples of healthy men obtained at infertility clinics were pooled. The material was centrifuged at 3000 rpm for 10 min at 4°C. The pellet containing sperms was discarded. The supernatant was mixed with four volumes of freshly distilled chilled ethanol, allowed to settle overnight at 4°C, and then centrifuged at 3000 rpm for 30 min to separate precipitated proteins. The precipitate was washed with chilled acetone followed by chilled ether, and finally air dried to yield the crude extract which served as the starting material for purification of the protein.

The crude extract powder was dissolved in 100 ml 0.05 M sodium deuteroacetate buffer, pH 4.0. Deuterated buffer was used to avoid the interference of resonances arising from the residual acetate in the NMR spectrum. Fractionation of the crude extract was effected on a Sephadex G-100 column (110 cm × 5 cm) (Pharmacia) using 0.05 M sodium acetate, pH 4.0 for equilibration and elution. Fractions monitored at 280 nm were collected at a flow rate of 1 ml/min and pooled on the basis of the elution profile. The active fraction from sephadex G-100 chromatography was concentrated by lyophilization and equilibrated by dialysis with tris-HCl buffer (0.05 M), pH 8.0. About 1 g of immunoactive material was loaded on a DEAE cellulose column (30 cm × 2.5 cm). The column was first washed with tris buffer to remove the unadsorbed proteins till it showed negligible OD at 280 nm using a flow rate of 0.5 ml/min. The adsorbed proteins were then eluted with Tris buffer containing 0.0–0.2 M sodium chloride continuous gradient. The immunoactive fractions were further purified by HPLC on a gel permeation preparative column (Ultropack TSK G-2000 SWG; 21.5 mm × 600 mm) with an exclusion limit of 200,000 daltons. The proteins were eluted with 0.05 M acetate buffer, pH 4.0 at a flow rate of 3 ml/min. The HPLC purified material was dialysed and lyophilized. The activity of HSPI was checked by radioimmunoassay[8].

Two-dimensional (2D) NMR experiments were carried out on a Varian Unity plus spectrometer with a ^{1}H frequency of 600 MHz. The 2D experiments in ^2H_2O included two-quantum-filtered correlation spectroscopy (2QF-COSY)[9], three-quantum-filtered correlation spectroscopy (3QF-COSY)[10], clean total correlation spectroscopy (clean-TOCSY)[11] with a mixing time of 80 ms, and nuclear Overhauser enhancement spectroscopy (NOESY)[12] with a mixing time of 150 ms. Spectra in 90% H_2O + 10% ^2H_2O included WATERGATE NOESY[13] with a mixing time of 200 ms and clean-TOCSY with a mixing time of 100 ms.

In the previously reported HSPI sequence of 94 residues all amino acid residues except alanine are present. The number of various amino acids is as follows: Gly: 5, Val: 6, Leu: 2, Ile: 6, Asp: 5, Asn: 5, Cys: 10, Ser: 7, Thr: 8, Glu: 8, Gln: 2, Met: 1, Arg: 2, Lys: 11, Pro: 5, Phe: 2, Tyr: 4, His: 1, Trp: 2. HSPI is particularly rich in lysines and cysteines. Though the occurrence of 11 lysine residues in a protein of 94 amino acids is not uncommon, the number of cysteine residues[10] is much higher than the statistical average for proteins of similar size. The spin systems of most of these residues have been identified using 2D NMR spectra recorded in ^2H_2O solution, which has been followed by sequence-specific resonance assignments using standard strategies[14]. The procedures involve identifying individual amino-acid spin systems using J-correlated spectroscopy followed by detection of sequential cross peaks arising from short distances (d_{NN}, $d_{\alpha N}$, $d_{\beta N}$ etc.) in the NOESY spectrum[14]. A relatively large percentage of unique dipeptide segments in HSPI, makes sequence-specific ^{1}H-NMR assignments much simpler. Search for a possible ambiguity in the primary sequence at position 93 started from the sequence-specific assignment of the two tryptophans (Trp32 and Trp92). In the NOESY spectrum Trp N(ε^1)H proton is easily identifiable because of its inherent chemical shift which is most down-field shifted (~ 10.3 ppm) in the ^{1}H spectrum (Figure 2). Two distinct nOes are observed for Trp N(ε^1)H arising from C(δ^1)H and C(ζ^2)H of the indole rings. The distinction between the two protons has been achieved from the observation of N(ε^1)H–C(δ^1)H cross peak in the TOCSY spectrum in H_2O (not shown here). N(ε^1)H and C(ζ^2)H are not J-coupled hence do not show mutual interaction in the TOCSY spectrum. However, since the inter-proton distance is 2.84 Å one observes strong nOe between these protons which helps in the identification of C(ζ^2)H (Figure 2). From the knowledge of C(ζ^2)H position,

	1 5		32		42		92		94	
HSPI	S C Y F I	---	W Q T D N C E	T C	T C	---	W	G	I	COOH
PSP-94	S C Y F I	---	W Q T D N C E	T C	T C	---	W	I	I	COOH
β-MSP	S C Y F I	---	W Q T D N C E	P T	T C	---	W	I		COOH
IgBF	S C Y F I	---						I	I	COOH

Figure 1. Amino acid sequence of HSPI and related proteins isolated from human seminal plasma.

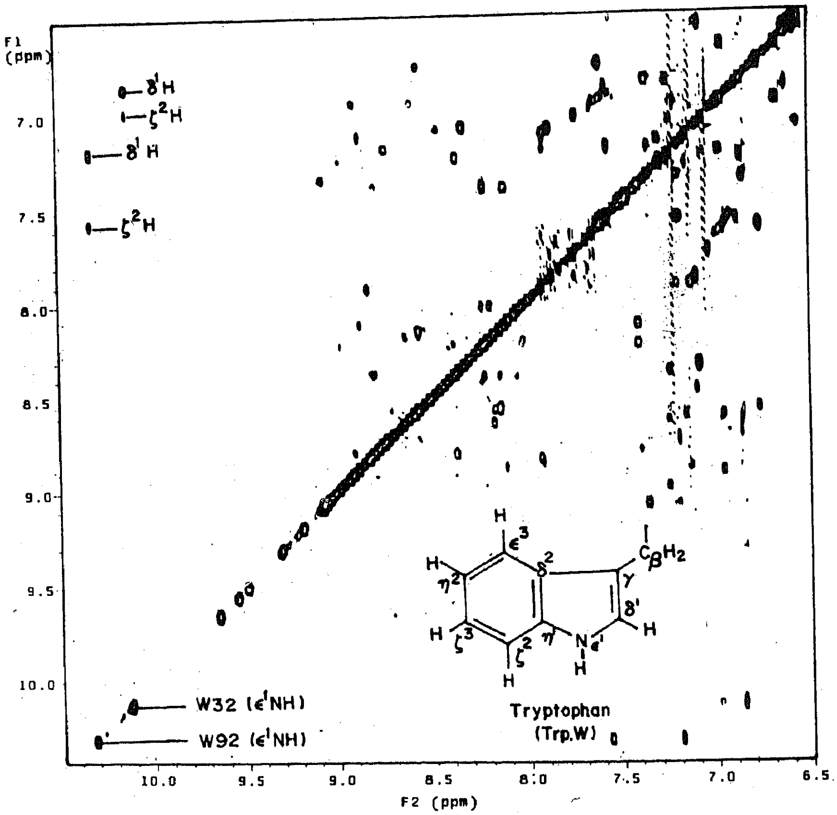

Figure 2. Selected region of the water-gated NOESY spectrum of HSPI recorded in 90% H_2O + 10% ^2H_2O at 20°C and pH 3.0. This shows the nOe connectivities from the side chain N(ε^1)H of two tryptophans to their respective C(δ^1)H and C(ζ^2)H protons. Experimental parameters: $t_1$max = 37.5 ms; $t_2$max = 256 ms; recycle delay = 1 s, 160 scans/t_1 increment. TD = 600 and 4096 along t_1 and t_2 dimensions, respectively. The data were multiplied with sine bell window functions shifted by $\pi/4$ and $\pi/8$ along t_1 and t_2 axes, respectively and zero filled to 1024 data points along t_1 dimension prior to 2D-FT. Digital resolution along ω_1 and ω_2 corresponds to 7.8 and 3.9 Hz/pt, respectively. The inset shows the indole ring of tryptophan with the standard nomenclature used for numbering the hydrogen atoms.

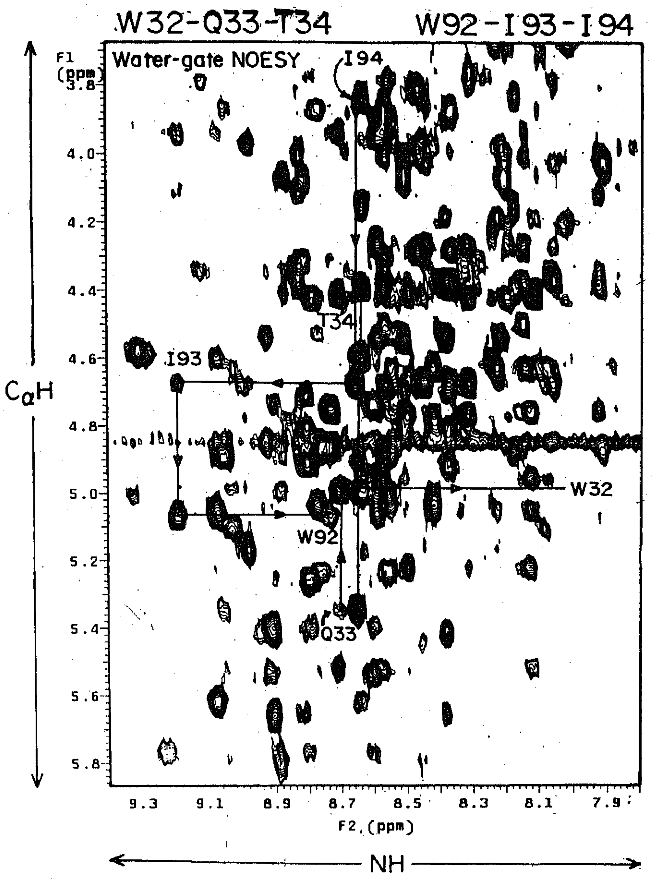

Figure 3. Finger-print region of the WATERGATE NOESY showing sequential connectivities (solid lines) for the protein segment Trp32–Gln33–Thr34 and Trp92–Ile93–Ile94.

the rest of the indole ring protons $C(\zeta^3)H$, $C(\eta^1)H$ and $C(\varepsilon^2)H$ have been assigned from the TOCSY spectrum in ^2H_2O (not shown here). We could identify both sets of indole proton resonances arising from the two Trp residues. Further, the intra-residue nOes $C(\delta^1)H$ to $C(\alpha)H$ and $C(\beta)H$ protons and $C(\alpha)H$ to NH (backbone) allowed complete identification of the remaining protons of the tryptophans. With this knowledge, nOe cross-peaks corresponding to sequential distances $d_{\alpha N}$, $d_{\beta N}$ and d_{NN} between neighbouring amino acid residues were assigned. This led to assignment of the glutamine and isoleucine spin systems (Figure 3), as sequential neighbours of Trp32 and Trp92, respectively. Sequential connectivities of Gln33 and Ile93 with Thr34 and Ile94, respectively confirm these assignments (Figure 3). The fact that segments Trp32–Gln33–Thr34 and Trp92–Ile93–Ile94 are unique in the polypeptide primary sequence, substantiates the sequence-specific resonance assignment. Thus, it could be unequivocally established that the amino acid at position 93 is Ile and not Gly as reported earlier. This is further supported by the identification of only four Gly residues in the COSY spectrum instead of five expected, if the amino acid at position 93 was Gly. Such a sequence correction suggests that HSPI and PSP-94 are one and the same protein.

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Liv.100 pretreatment inhibits the CCl_4 induced lipid peroxidation in rat liver – An *in vitro* study

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The protective effect of Liv.52 and Liv.100 against *in vitro* peroxidation induced by carbon tetrachloride in rat liver homogenate has been studied. Lipid peroxidation was measured in terms of thio-barbituric acid reactive substances (TBARS), and reduced glutathione was also assayed. Addition of the two polyherbal formulations, Liv.52 and Liv.100, inhibited the peroxidation effect of carbon tetrachloride in a dose and time-dependent manner. The results suggest on the antioxidant potential of Liv.52 and Liv.100. Liv.100 is a new ayurvedic formulation.

LIPID peroxidation is a natural phenomenon and occurs on exposure to oxygen[1]. Active oxygen molecules such as superoxide radicals ($O_2^{\cdot -}$, ^-OOH) and hydroxyl radicals ($OH^{\cdot}$) play an important role in the inflammation process after intoxication by carbon tetrachloride, ethanol or carrageenan[2–5]. These radicals react with cell membranes, induce lipid peroxidation and have been implicated as important pathologic mediators in many clinical conditions[6–8]. This communication is based on the potential of the two ayurvedic formulations, Liv.52 and Liv.100. Liv.52 is commercially available in India and has been reported to be clinically effective in treating hepatotoxicity and a wide range of liver diseases[9–11]. Recent reports also suggested the protective effect of Liv.52 against radiation-induced chromosome damage[12,13] (lipid peroxidative changes) in mouse bone marrow. Therefore, an attempt was made to study the effect of the new ayurvedic formulation Liv.100 on lipid peroxidation in rat liver homogenate exposed to carbon tetrachloride challenge and compared with that of Liv.52. Liv.100 is also a product of Himalaya Drug Co, a polyherbal preparation composed of *Cichorium intybus*, *Solanum nigrum*, *Phyllanthus amarus*, *Piccorhiza curra* and *Embelica ribes* in the ratio 5 : 3.75 : 2.62 : 2 : 1. These herbs are individually reputed for their hepatoprotective nature[9,11,14,15].

Male albino rats of wistar strain (100 ± 125 g) maintained on a standard pellet diet supplied by M/s Hindustan Lever Ltd (Bangalore, India) and water *ad libitum* were used for the experiment.

Rats were sacrificed by cervical dislocation and liver was immediately excised. A homogenate (1 g, w/v) was prepared using phosphate buffer saline (PBS), pH 7.4, in cold. It was centrifuged at 2000 *g* for ten minutes. Supernatant was collected and finally suspended in PBS. It contained approximately 0.8–1 mg protein in 1 ml of suspension. This was used to perform the *in vitro* experiment.

To establish the role of Liv.52 and Liv.100 on carbon tetrachloride-induced lipid peroxidation[16], different concentrations of Liv.52 and Liv.100 were added to 3 ml of liver homogenate, mixed and incubated at 37°C for 20 min. This was followed by the addition of carbon tetrachloride (1 mM), mixed gently and further incubated at 37°C for 25 min to induce the production of TBA-reactive substances (TBARS). The control group contained buffer and different concentrations of Liv.52 and Liv.100. Lipid peroxidation was measured in terms of TBARS as described by Yagi *et al.*[17] with slight modification and reduced glutathione was measured by the method of Ellman[18]. Protein was determined by the method of Lowry *et al.*[19] using bovine serum albumin as standard.

The results presented in the paper are the mean ± SD of six animals. Level of significance has been evaluated by using Student's *t*-test.

The reaction mixture that contain carbon tetrachloride *per se* resulted in a significant increased ($P < 0.001$) formation of TBARS (Table 1). Addition of the two drugs proved their protective nature against carbon tetrachloride-induced lipid peroxidation. A significant ($P < 0.001$) dose-dependent effect was afforded by the drugs, and did not show any augment in TBA-reactive substances up to 6.6 mg/ml.

Figure 1 shows the effect of Liv.52 and Liv.100 (1 mg/ml) on reduced glutathione (GSH). In normal condition, GSH content decreased after 20 min as an outcome of auto-oxidation, but in the (1 mg/ml) drug-treated group, the rate of lowering was found to be minimum and the level was maintained up to 40 min. This effect was seen even in the presence of 0.25 mM carbon tetrachloride. The effect of the drugs was found to be appreciable in maintenance of the levels of glutathione at normal (Figure 1).

In the present study, carbon tetrachloride was used as a peroxidative agent, and has been justified to produce free radicals or deplete antioxidant defenses. Carbon tetrachloride is converted to a free radical by hepatic cytochrome P-450.

$$CCl_4 \xrightarrow{\text{Cytochrome P-450}} Cl^{\cdot} + CCl_3^{\cdot}.$$

This radical reacts with oxygen to give a peroxy radical $CCl_3^{\cdot} + O_2 \rightarrow CCl_3O_2$ which is a good inhibitor of lipid peroxidation. Consequently, there is depletion of antioxidant, especially glutathione. Loss of glutathione causes secondary oxidative damage, which can contribute to hepatic failure.

The reason for having employed two different concentrations of CCl_4 (1 mM and 0.25 mM) for the assay

Table 1. Protective effect of Liv.52 and Liv.100 on carbon tetrachloride (1 mM)-induced lipid peroxidation in rat liver

Drug concentration (mg/ml)	0	0.8	1.6	3.3	6.6
Liv.52					
Without CCl_4 'TBARS' nM/100 mg protein	117.08 ± 4.71	116.99 ± 4.20	116.83 ± 3.74	116.69 ± 3.94	116.37 ± 3.62
With CCl_4 'TBARS' nM/100 mg protein	231.85 ± 4.65*	222.90 ± 3.11*	171.97 ± 3.32*	158.47 ± 2.98*	127.36 ± 3.05*
Liv.100					
Without CCl_4 'TBARS' nM/100 mg protein	117.11 ± 4.69	116.78 ± 2.26	116.51 ± 2.35	116.35 ± 2.02	116.24 ± 2.08
With CCl_4 'TBARS' nM/100 mg protein	231.91 ± 4.58*	189.37 ± 1.97*	143.20 ± 2.60*	125.16 ± 2.23*	116.30 ± 2.01*

Values are mean ± SD of 6 animals in each group.
*$P < 0.001$.

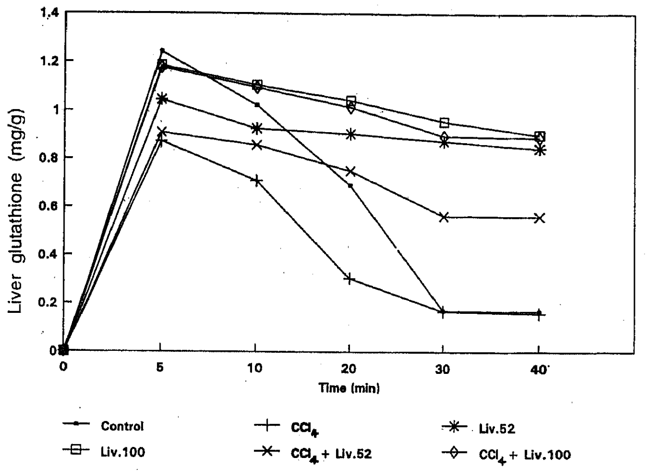

Figure 1. Effect of carbon tetrachloride (0.25 mM), and Liv.52 and Liv.100 (1 mg/ml) on the changes in reduced glutathione content in rat liver. Each point represents mean value of six animals.

of lipid peroxidation (TBARS) and reduced glutathione, is that at higher doses of 1 mM concentration, it released detectable amounts of TBARS but reduced the GSH level (almost to zero). This showed that the oxidation of GSH is followed by lipid peroxidation and therefore, quantitation of reduced GSH would be more sensitive test than TBARS formed.

Carbon tetrachloride-induced peroxidation of lipid in liver homogenate was found to be suppressed by the addition of Liv.52 and Liv.100. The effect was almost completely, inhibited by Liv.100 when compared to Liv.52.

The present results show the dose and time-dependent protective response afforded by Liv.52 and Liv.100 against carbon tetrachloride-induced lipid peroxidation. These results suggest that the drugs act at the lipid peroxide scavenging system through enzyme control or otherwise, by enhanced supply of reduced glutathione[20] that suppressed the deleterious process.

However, the findings indicate that the formulations Liv.52 and Liv.100, contain certain substances capable of preventing lipid peroxidation. Polyphenolic constituents[14,21] of the herbs in the above ayurvedic formulations may be considered to be antioxidants able to react with numerous radicals in cell-free systems, including oxygen and hydroxylic ($OH^{\bullet}$) radicals forming more stable and less reactive compounds[22,23].

To conclude, it is hoped that the beneficial antioxidant nature of Liv.52 and Liv.100 may be applied clinically in the future only after considerable *in vivo* trial with the drugs.

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Isotopic compositions of boron in sediments and their implications

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The abundance and isotopic compositions of boron in sediments from the salt lakes of Qaidam Basin, China have been determined by thermal ionization mass spectrometry of cesium borate. The results show large variations in the isotopic compositions of boron. The observed variations of δ^{11}B, ranging from – 25.2‰ to + 26.08‰ (relative to NBS SRM 951) indicate both an enrichment and depletion in the lighter isotope (^{10}B) in the lake sediments. The lower δ^{11}B values of sediments are mostly closer to the boron isotopic compositions of minerals. Such low δ^{11}B values are attributed to the presence of borates, ulexite and other carbonate minerals in sediments of the salt lakes of Qaidam Basin.

BORON is one of the major elements in seawater[1] and is significantly involved in marine biogeochemical cycles[2,3]. Recent studies have shown that the isotopes of boron act as excellent tracers of geochemical processes[4–6]. The differences in the behaviour of its two stable isotopes, ^{10}B and ^{11}B, result in the isotopic fractionation of boron during its biogeochemical cycling[7] and the extent of this fractionation provides insights into the various processes involved.

Although the isotopic studies of boron are being carried out for the past 2–3 decades, the difficulties involved in the accurate measurement of boron isotopic ratios led to a slow progress in this area. However, the recent advancements in the analytical techniques[8,9] have provided impetus to studies on isotope geochemistry of boron. The isotopic measurements by these techniques have shown wide variations in boron isotopic ratios in nature[10] and indicated distinct differences in the isotopic compositions of boron between the marine, non-marine and terrestrial samples and between seawater, sediments and the upper oceanic crust[11,12].

The hypersaline environments experiencing intense evaporation are excellent sites for isotopic studies of elements as considerable isotopic fractionation occurs during evaporation and precipitation in the brine[13]. The present study is based on the boron isotopic measurements carried out in sediments of the salt lakes of Qaidam Basin, China, from October to December, 1992 to understand their variation and their geochemical implication.

The salt lakes of Qaidam Basin are situated to the north of the Kunlun Tangula mountains in the northwestern part of Qinghai Plateau at an altitude of over 2600 m above sea level. This terrestrial basin (Figure 1), evolved during the Jurassic period[14], is typically a continental potassium-bearing basin with an area of 12×10^4 km^2. It is surrounded by the Altun mountains in the northeast and Kunlun mountains in the south. The streams draining from these snow-capped mountains terminate in a series of saline lakes at the margin of the basin.

The basin experiences a typical continental climate with difference in day and night temperatures, reaching as high as 30°C. The climate is arid and cold with an annual precipitation less than 50 mm and annual evaporation far exceeds precipitation. Due to the low air temperatures, high aridity and strong winds, the waters in these lakes have turned into brines.

Sediments were collected from 22 salt lakes in the Qaidam Basin (Figure 1) during 7 July–10 August, 1992. After collection, the brine was drained out and the sediments were oven dried at 60°C. The dried sediments were finely powdered and sieved through a standard 100 mesh. A known quantity of the sieved sample was treated with a minimum quantity of dilute HCl (< 0.1 N). The acid-washed sediments were filtered and the filtrate was preserved for isotopic measurements. The residue was then washed with distilled water and again filtered. The filtrates were mixed together and made up to 50 ml. Boron contents in the filtrates were determined through mannitol-alkalimetry titration[15]. A known quantity of the filtrate was used for the extraction of boron by ion-exchange for isotopic measurements[16].

A mixture of weakly basic anion exchange resin in HCO_3^- form and a strongly acidic cation exchange resin in the H^+ form was poured into a special glass column. The boron-containing solutions were slightly acidified with HCl before passing through the column. After shaking for 7–10 min, boron was eluted with 60 ml high-purity water. The yield of boron recovered from each sample was more than 98%. After elution, the ^{11}B/^{10}B ratio was measured by a mass spectrometer, model VG 354, based on thermal ionization mass spectrometry of cesium borate using graphite as a substrate

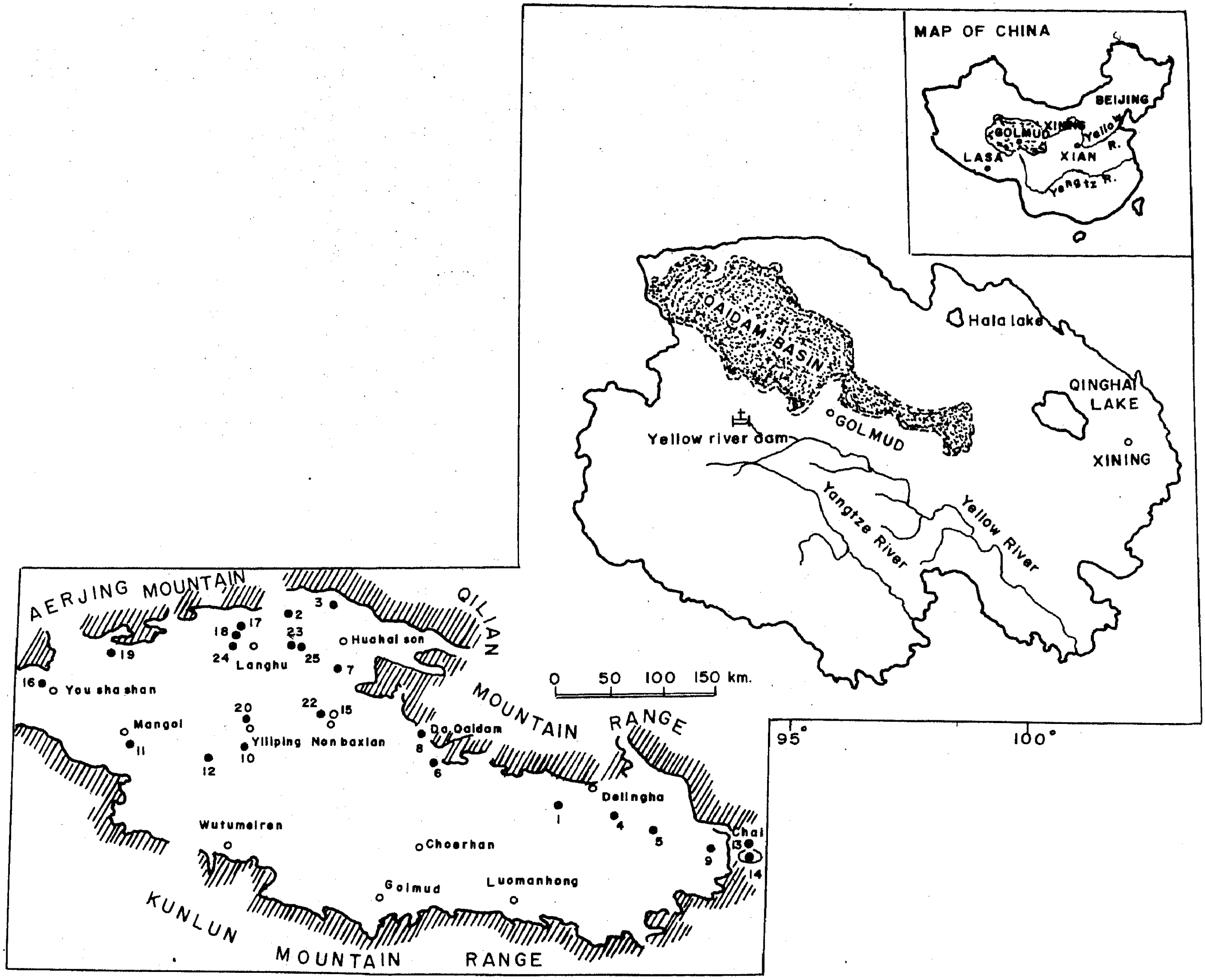

Figure 1. Location of Quidam Basin and the salt lakes in Quidam Basin, China.

for loading[8]. The $^{11}B/^{10}B$ ratio of NBS SRM 951 was first obtained and was found to be 4.04362 ± 0.0011. The isotopic ratios of the samples were then expressed as del values (per mil deviation, $\delta^{11}B$, relative to NBS SRM 951 Boron Standard value of 4.04362), according to the equation

$$\delta^{11}B(\text{sample}) = [(^{11}B/^{10}B)\text{sam}/(^{11}B/^{10}B)\text{std} - 1] \times 1000,$$

with a precision of 0.023% (95% confidence limit) for the standard solution while for the samples, the precision was 0.03%.

The $^{11}B/^{10}B$ ratios of sediments varied from 3.9416 to 4.1491 with a mean of 4.0431. The $\delta^{11}B$ values were found to range from $-25.21‰$ to $+26.08‰$ (Table 1).

The main components of the salt lake sediments were halite, gypsum and clay minerals. The clay minerals constituted 5–20% of the sediments; of these, the most abundant were illite (58–78%) and kaolinite (11–42%) (Table 1).

The isotopic compositions of boron showed low (negative) values for most of the sediments from the salt lakes of Qaidam Basin. Even the higher (positive) values of $\delta^{11}B$ of sediments were much lower than those of the overlying brines, collected simultaneously from the respective salt lakes[17]. This reveals the effect of isotopic fractionation of boron wherein the lighter isotope, ^{10}B, is preferentially removed from the overlying brines to sediments. The fractionation of boron has been attributed to its tetrahedral species, $B(OH)_4^-$ which is enriched with ^{10}B (ref. 4). Due to the greater affinity of ^{10}B for solids, the borate anion gets preferentially adsorbed onto solid substances when dissolved boron comes in contact with solids[18].

Table 1. Abundance and isotopic compositions of boron and the clay minerals in Salt Lake sediments

Location (lakes)	Boron (mg/kg)	Clay mineral (%)			$^{11}B/^{10}B$	$\delta^{11}B$
		Illite	Kaolinite	Chlorite		
Tuosu	0.18	69	31	–	4.0232	– 5.05
Xiaosugan	0.04	78	22	–	4.0280	– 3.86
Yiligou	0.05	NA	NA	NA	4.0388	– 1.20
Xiao Qaidam	0.78	72	28	–	4.0012	– 10.48
Zhongmahai	0.44	NA	NA	NA	4.0003	– 10.71
Da Qaidam	2.45	74	36	–	4.0549	+ 2.78
Qaikai	0.33	77	23	–	4.0350	– 2.13
Xitaije Ner	1.42	58	42	–	4.0356	– 1.99
Mangai	0.45	NA	NA	–	4.0752	+ 7.80
Tar Din	0.80	NA	NA	NA	3.9416	– 25.21
Keke	0.17	68	32	–	4.0631	+ 4.82
Nanbaxian(I)	0.16	NA	NA	NA	4.0257	– 4.43
Gasikule	0.42	72	28	11	4.0971	+ 13.21
Kunteyie(I)	0.60	NA	NA	NA	4.0246	– 4.71
Potassium(I)	0.62	NA	NA	NA	3.9781	– 16.20
Dalang Tan(I)	1.30	72	11	17	4.1491	+ 26.08
Balunmahai	0.07	70	30	–	4.0329	– 2.65
Nu Lang	0.88	NA	NA	NA	4.1299	+ 21.35
Potassium	0.46	NA	NA	NA	4.1104	+ 16.51
Zhinu	0.83	69	31	–	4.1250	+ 20.12
Jiahu	0.62	68	32	–	3.9781	– 16.20
Dezong	0.47	NA	NA	–	4.0003	– 10.71

I: Interstitial brine.
NA: Not analysed.

Various factors such as pH, temperature, ionic strength, boron concentration of solution and the nature of minerals affect the fractionation of boron[4,7,12]. The most important factor affecting the speciation of boron is pH as it controls the relative proportion of the two dominant species – $B(OH)_3$ and $B(OH)_4^-$ (ref. 19). The abundance ratio of $B(OH)_3$ to $B(OH)_4^-$ in turn influences the $\delta^{11}B$ in sediments and minerals. Ingri *et al.*[19] showed that within the pH range of 5–12, the $B(OH)_3$ to $B(OH)_4^-$ ratio decreases as the proportion of $B(OH)_3$ decreases with increasing pH. The pH values of the overlying brines in these lakes[17] were within a comparable range (4.43–8.9). Such a range leads to variable mole fractions of $B(OH)_3$ in brines and thus to different $^{11}B/^{10}B$ ratios and $\delta^{11}B$ values in sediments.

The $\delta^{11}B$ values of the salt lake sediments in this study (Table 1) are much lower than those reported for the marine and nonmarine sediments (the nonmarine environment in this case refers to the freshwater environment). However, the salt lakes of Qaidam Basin represent an evaporative environment. In such an environment, boron is not significantly removed by precipitated salts but remains in the brine and gets concentrated in evaporitic clay minerals rich in magnesium, as the tetrahedrally coordinated boron[20,21]. The studies on the geochemistry of boron[3] have shown that the adsorption of boron takes place on sediment particles and the clay minerals are very significant for adsorption. This adsorption leads to isotopic fractionation of boron[22].

The clay minerals of the salt lake sediments comprise 58–78% illite, 11–42% kaolinite and 11–17% chlorite (Table 1). The adsorption of boron on these minerals therefore contributes to the lowering of $\delta^{11}B$ values observed in this study. Apart from this, boron is also taken up by boron minerals and the isotopic composition of borate salts is dependent on their atomic configuration and the proportion of boron species in the mineral[23]. The boron (as borate) content of some of its minerals has been given as (100% BO_4) boracite, (66% BO_4) colmanite, (60% BO_4) ulexite and (50% BO_4) borax. These minerals are always depleted in the heavier isotope ^{11}B relative to that in the parent brines[24].

Similarly, different minerals are found to have different boron isotopic composition values, e.g. authigenic marine silicates (– 38‰); the adsorbed boron onto marine sediments (–25‰) and the modern marine biogenic carbonates (– 7 to – 25‰). The boron isotopic compositions of some of the boron minerals are: – 4‰ (borate); – 12.8‰ (ulexite) and from – 17.24 to – 23.67‰ (pinnoite)[12,18,24]. As seen from our values in Table 1, the $\delta^{11}B$ values of the sediments from the Tuosu lake (– 5.05‰), Nanbaxian lake (– 4.43‰) and the Kunteyi lake (– 4.71‰) are closer to those of the borates (– 4‰). So also, the $\delta^{11}B$ values of the sediments from potassium lake (– 16.2‰) and the Kunteyi lake (– 16.20‰) are closer to that of ulexite (– 12.8‰) while the $\delta^{11}B$ values of many other lake sediments fall in the range for carbonates.

The main boron minerals found in these lake sediments are ulexite, pinnoite, borax and other potash minerals of chloride and sulphate type. The chloride minerals are carnallite, sylvite, halite and bischofite, whereas the sulphate type are mirabilite, picromerite and polyhalite which are always found in association with epsomite, bloedite, mirabilite or gypsum[25–27]. Oi *et al.*[23] observed that the minerals of marine origin have higher $^{11}B/^{10}B$ ratio and higher $\delta^{11}B$ value than those of the nonmarine origin and the $^{11}B/^{10}B$ ratios of minerals from the same geologic origin depends upon the crystal structure. A correlation exists between the crystal structure of the mineral and its $\delta^{11}B$ values. They attributed it to the reduced partition function ratios (RPFRs) of the two species of boron based on the general theory of isotope effects. The RPFR of the $B(OH)_3$ species is larger than that of the $B(OH)_4^-$ and the mineral having the higher $B(OH)_3/B(OH)_4^-$ ratio has larger $^{11}B/^{10}B$ ratio and larger $\delta^{11}B$ value at any temperature. Thus it has been realized that the abundance ratio of $B(OH)_3/B(OH)_4^-$ in the solution has a profound effect on the boron isotopic composition of the mineral formed. The varying pH of the overlying brines of these salt lakes (4.43–8.90) may therefore result in differences in the mole fraction of $B(OH)_3$ and $B(OH)_4^-$ giving rise to different values of $\delta^{11}B$ for the minerals. So, the slight variation in $\delta^{11}B$ values observed in our study compared to that of the boron minerals given above, is due to varying pH of the brines of the salt lakes and different origin. These $\delta^{11}B$ values can therefore be attributed to the presence of borates, ulexite and other carbonate minerals and as such assume greater importance in the exploration of minerals and the characterization of environmental samples.

In conclusion, the boron isotopic compositions of the salt lake sediments indicate the presence of borate, ulexite and other carbonate minerals. During the formation and transformation of minerals in sediments (particularly boron minerals), substantial element exchange takes place. This leads to the isotopic fractionation of boron. So, the boron isotopic study of sediments can give an understanding of sediment diageneses. Measurement of the boron isotopic composition of sediments by mass spectrometry can serve the dual purpose of detecting the minerals and the isotopic composition of the sample.

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PERSONAL NEWS

Professor Paul Erdős (1913–1996)

An obituary

Professor Paul Erdős (pronounced Airdosh) was born in Hungary on 26 March 1913. It is reported that even at the age of 3, he had invented negative numbers by himself when he set out to answer the question. How much is 250 degrees below 100 degrees? His answer was 150 degrees below zero. A few years later he amused himself by finding out how long does a train take to reach the sun. He had two elder sisters both of whom died of scarlet fever a few days before he was born. So his mother took very good care of him. He never got married and his mother helped him by taking care of his publication list and so on. She travelled with him wherever he went and Erdős was extremely sad after she passed away. His was a life dedicated completely to mathematics. He was so prolific that he had more than 1500 important papers to his credit. He was so versatile that he could contribute significantly to (almost) any mathematical problem. No wonder if, I call his contributions as 'Erdőssian Mathematics'. Some people who found that it was difficult to follow his work, say that he does not do mathematics. For some time I thought that such people invented the concept 'Erdős Number' to make fun of the fact that he writes too many joint papers (the Erdős number of a collaborator with him is 1, that of a collaborator's collaborator is 2 and so on). But later on I found (from A. Ivić) that this concept was invented by Erdős himself (for some purposes). Mathematicians would look upon Erdős as god himself and consider a close association with him as an indication of their merit (close association with him will mean a small Erdős number) and greatness. Erdős' mathematics, so terse and at the same so attractive, is described rightly by Ernst Straus (who worked both with Einstein and Erdős) as follows:

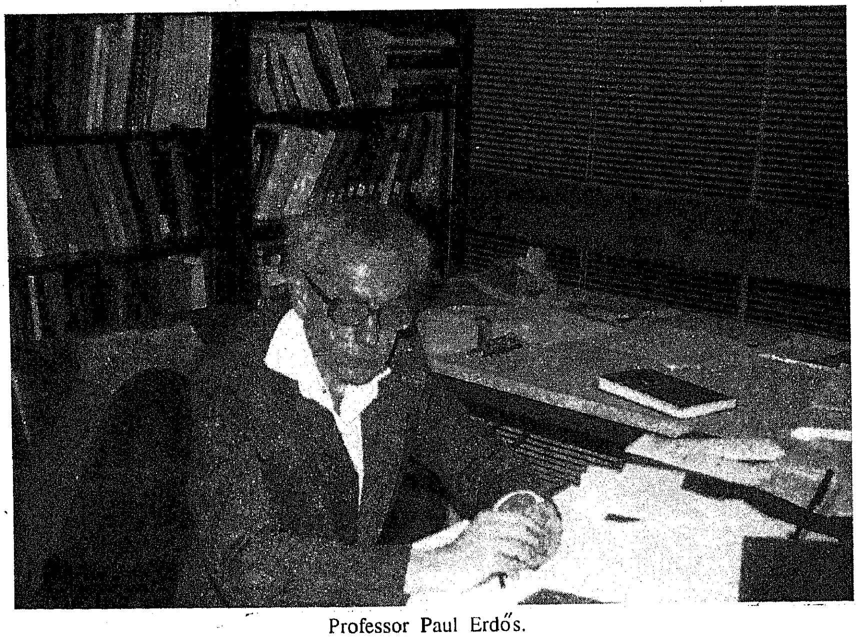
> 'In our century in which mathematics is so strongly dominated by "Theory Doctors" he has remained the prince of problem solvers and absolute monarch of problem posers.'

Looking at the vast number of papers which he has written one cannot help comparing him to L. Euler and looking at the depth and attractiveness of Erdőssian mathematics one cannot deny that he is 'in some sense' the greatest mathematician of this century. Judged from whatever angle I agree with R. Graham that he is certainly amongst the top ten in this century. He had 458 collaborators (that is those with Erdős number 1) and about 4500 with Erdős number 2 and about 100 papers will be published by his collaborators after his death. I am proud to say that I have Erdős number 1 (about which I will say in a later section). On the whole I can say that many subjects will emerge by his contributions.

Some reminiscences

I had heard of Erdős as an extra ordinary mathematician (and I had looked at some of his papers early in my research career) and I was longing to meet him (although I never expected to have that golden opportunity). In 1970–71 I was a neighbour of C. Hooley (at Princeton, Institute for Advanced Study) and he came to know that Erdős will be in New York Academy of Sciences for a few days. He telephoned Erdős and arranged for a meeting with him. He drove me to New York and we were very happy to meet Erdős and his mother. Erdős gave a lunch party to us during which he introduced us to his mother. Thereafter I used to write to him frequently and he promptly replied all my letters. At this point I would like to say that no matter whether you are amateur or a professional he would promptly reply your letters (he wrote about 15 to 20 letters a day). Every time it would be a friendly letter and suggestion of some mathematical problems. Another point about Erdős address: he never used to stay at any place for more than a week and he has travelled practically throughout the world. I always wrote to his Hungarian address. Hungarian Academy used to redirect my letters to his address. The letters would have to travel throughout the world to reach him. What is very amazing is that he remembered what he wrote to people. Each letter to them would contain suggestion of several problems, contributions for collaborative work besides enquiring welfare of family members and friends.

He has visited TIFR twice. On one occasion guest house was (luckily for us)



Professor Paul Erdős.

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*This obituary is based on two reports on this topic (namely obituary) one from Brestilov Novak and another from Aleksander Ivić. In addition, it contains something about Professor Erdős which I report from my experience.

not available. We (my family and I) had him as our guest at our residence. We felt very much honoured and we will keep it as a valuable treasure in our minds the memory of his visit to us. He would work till 2 at night drinking frequently a cup of black coffee. His habit was to work almost round the clock. (Also he liked the food in our house.) Another friend in TIFR closely connected with him is N. M. Singhi.

Encouragement to E. Szémerédi

As has been said before, Erdős used to encourage difficult attractive research very much. He used to state such problems and announce prizes for solutions. Many youngsters have received prizes from him. I once spoke to him about the work of H. Maier and he told me that Maier had knocked off several prizes from him. (H. Maier, known for his highly original work on large and small differences between consecutive primes and so on, is at present a professor at ULM in Germany.) Once Erdős had proposed the following problem:

Let $k \geq 3$ be any fixed integer and N a variable integer. Let S_1 be maximal subset of integers from the set $\{1, 2, \ldots, N\}$ with the property that no k integers of S_1 are in arithmetical progression. Let $M = M(k)$ be the number of integers in S_1. Then

$$\lim_{N \to \infty} \frac{M}{N} = 0.$$

This was solved by E. Szémerédi in an outstanding paper. Erdős proposed him for Fields Medal. But much to the dismay of the world he was not selected for Fields Medal. Erdős was annoyed. But he did not keep quite. In the same gathering of IMU (International Congress of Mathematicians) he gave \$2500, a medal and a citation to Szemeredi. (Note also that \$2500 exceeds the amount of the Fields Medal.) No individual has struggled all by himself and upheld the cause of deep and attractive mathematics as Paul Erdős.

A mathematician with no home, no job, a great seeker after properties of $1, 2, 3, \ldots$, Erdős would travel from place to place bearing the torch of knowledge and he would propose problems, announce prizes and help people to progress in their work. (At this point one remark: He remembered references to many (important) papers including his own, so much so that he may be known as a mobile encyclopaedia. People may think that he travelled with a huge luggage but his only luggage was a half empty small size suit case.) I mention a few attractive problems of his with prizes.

(1) Let $1 = b_1, b_2, b_3, \ldots$ be any increasing sequence of integers with the property that

$$\sum_{n=1}^{\infty} \frac{1}{b_n} = \infty.$$

Then given any fixed integer k there exist $k(\geq 3)$ distinct integers b_ν $(\nu = n_1, n_2, n_3, \ldots, n_k)$ forming an arithmetical progression. (Prize \$20,000).

(2) Let p_n denote the nth prime. Then prove or disprove that

$$\lim_{n \to \infty} \frac{p_{n+1} - p_n}{\log n \ \log\log n \ \log\log\log n} > 0.$$

(Prize \$20,000).

He would also increase the prize from time to time if a problem remained unsolved for longer periods.

Some contributions of Erdős

It is practically impossible to touch upon all the ≥ 1500 papers of Erdős. A. Ivić remarked that he would pity the person who prepared the bibliography of all the papers of Erdős. (Suffice to say that all these papers involve considerable skill and originality.) I would single out three discoveries of Erdős.

(a) G. H. Hardy and J. E. Littlewood could prove that

$$\lim_{n \to \infty} \frac{p_{n+1} - p_n}{\log n} < 1$$

(that the above limit is ≤ 1 is a trivial consequence of the prime number theorem which asserts that $p_n(n \log n)^{-1} \to 1$ as $n \to \infty$) only on the assumption of a very difficult *unproved hypothesis* called Generalized Riemann hypothesis. Erdős succeeded in proving this without using any unproved hypothesis. He achieved it by a very ingenious use of Brun's Sieve.

(b) The prime number theorem stated just now was proved (by methods independent of theory of functions of one complex variable) by P. Erdős and A. Selberg independently of each other.

(c) For integers $n(\geq 2)$ let $\omega(n)$ denote the number of distinct prime factors of n (for our purpose it does not matter if the prime factors are counted with multiplicity). Let $f(n)$ be any positive function which $\to \infty$ as $n \to \infty$. Let $F(x)$ denote the number of integers $n \leq x$ with the property

$$|\omega(n) - \log\log n| \leq f(n) \sqrt{\log\log n}.$$

Then G. H. Hardy and S. Ramanujan proved (for the first time after conjecturing it) the result that

$$\frac{F(x)}{x} \to 1 \quad \text{as} \quad x \to \infty.$$

P. Erdős and M. Kac put forth the best of their talents and proved by an ingenious method the following result (which says a lot more about the theorem of G. H. Hardy and S. Ramanujan). Let C_1 and C_2 be any two real constants with $C_1 < C_2$. Let $F(x, C_1, C_2)$ denote the number of integers $\leq x$ with the property

$$C_1 \sqrt{\log\log n} \leq \omega(n) - \log\log n \leq C_2 \sqrt{\log\log n}.$$

Then

$$\frac{F(x, C_1, C_2)}{x} \to \left(\int_{-\infty}^{\infty} e^{-u^2} \, du \right)^{-1} \left(\int_{C_1}^{C_2} e^{-u^2} \, du \right).$$

(A passing remark: An Indian who has studied this theorem and worked out some variants of this theorem is A. Krishnaswamy.) Erdős–Kac theorem forms the foundation of a deep aspect of probabilistic number theory.

Encouragement to me and some others

Let u and k be positive integers and let $P(u, k)$ denote the greatest prime factor of $(u+1)(u+2)\ldots(u+k)$. One of the early interests of P. Erdős is what I call Sylvester–Erdős Theorem (Sylvester was the first to prove it and Erdős supplied a simpler proof). It states that

$$\min_{u \geq k} P(u, k) > k\,.$$

Note that this is a two-variable generalization of Chebyshev's theorem that $P(k, k) > k$ (which is nothing but the well-known Bertrand's postulate). Erdős also proved that

$$\min_{u > k^{3/2}} P(u, k) > \frac{1}{3}\, k \log k$$

provided k exceeds a large constant (we make from now on this hypothesis about k).

Independently of all this (not knowing this) I considered the special case $k = [u^{\frac{1}{2}}]$ and proved that $P(u, k) > u^{\lambda}$ with $\lambda = \frac{1}{2} + \frac{1}{13}$ by using Selberg Sieve and Vander–Corput sums. Erdős encouraged me to consider the case $u = [k^{\frac{1}{100}}]$ and things of that kind and I was led to prove that (apart from ε's δ's)

$$\min_{u \geq k^{3/2}} P(u, k) > k \log k\,.$$

However it was R. Tijdeman who sharpened (by Halberstam–Roth method, completely different from mine) the lower bound to $2k \log k$. Using (A. Baker's method on linear forms) myself and T. N. Shorey (improved Tijdeman's result to the fact that 2 can be replaced by any constant) and (using I. M. Vionogradov's methods) M. Jutila could improve it further by combining it with myself T. N. Shorey procedure. The final outcome is

$$\min_{u \geq k^{3/2}} P(u, k) > \mu k \log k \log\log k (\log\log\log k)^{-1}\,,$$

where $\mu > 0$ is a positive constant (we have the result $p_{n+1} - p_n = O(p_n^{5/8})$ which implied that if $k \leq u \leq k^{3/2}$, $P(u, k) > u$). This is a very good achievement (the best known to-day) due to myself, T. N. Shorey and M. Jutila. Needless to say that Erdős was encouraging all the time at all stages of our work.

Another problem concerns is generalization of Mirsky's result that the number of square free numbers s and such that

$$2n = p + s \; (p - \text{prime})$$

is asymptotic to a constant (>0) times $n(\log n)^{-1}$. Myself and G. J. Babu considered the number of solutions in integers (with certain property be stated below) satisfying

$$2n = p + s$$

is asymptotic to a constant (>0) times $n(\log n)^{-1}$. The property is this: Let $b_1, b_2, \ldots$ be away increasing sequence of positive integers (with $b_1 \geq 2$) such that for any two distinct integers i, j we have $(b_i, b_j) = 1$ and

$$\sum_{j=1}^{\infty} (\varphi(b_j))^{-1} < \infty\,.$$

(The numbers s are assumed to be not divisible by any b_j.) We had to introduce some further conditions and Erdős removed practically all the conditions. The result was a joint paper by myself, G. J. Babu and Erdős. In another joint paper we considered also further relaxation of conditions and obtained the lower bound of the same order instead of an asymptotic formula. All this was possible only because of various letters from Erdős.

I should mention that he has joint papers with T. N. Shorey, R. Balasubramanian and a complete account of these contributions would require a longer article.

Erdős was an honorary fellow of the Hardy–Ramanujan Society besides having many other honours throughout the world. He was on the editorial boards of many journals in the world (*Acta Arithmetica*, *Journal of Combinatorics*, *Bulletin of the Calcutta Mathematical Society*, *Journal of the Indian Mathematical Society*, and so on). His service to mathematics is so great that we feel that the world is not the same without him. In fact there exists in the minds of some of his followers a fact that they belong to the Erdős International Academy of Mathematics, Erdős International University and so on. Professor A. Ivić is one of them. Erdős passed away on 20 September 1996 in Warsza, Poland.

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Annual Review of Pharmacology and Toxicology 1996. Arthur K. Cho (ed.). Annual Reviews Inc., 4139 El Camino Way, Palo Alto, California 94303-0139, USA. Vol. 36. Price: USA $ 52, elsewhere $ 57. 719 pp.

The Annual Review of Pharmacology and Toxicology, like other annual reviews of medical sciences, has a long tradition of publishing good articles on selected topics and/or areas relating to different fields of medical sciences, thereby highlighting the important developments in different areas of research. This volume has 26 review articles that can be broadly classified into 8 groups: Prefatory chapters; Antimicrobial and antiviral chemotherapy; Cancer chemotherapy and immunopharmacology; Cardiovascular and endocrine pharmacology; Environmental and industrial pharmacology and toxicology; Mechanisms of drug actions, Neuro and behavioral pharmacology.

The volume begins with the first prefatory article written by Nobel laureate Sir James Black, who contributed to the discovery of propranolol and cimetidine. In this chapter, Black adumbrates the way his ideas about pharmacological analysis have developed over 40 years and explains concepts, models and techniques used in analysis and also a need for the future development. The central theme is how the development of simple mathematical model has assisted in the interpretation of drug action. The second prefatory article 'A career in toxicology' is interesting and useful to young toxicologists, who could learn from the varied exciting experiences of R. A. Neal. The author finally states that there is a distorted public perception of risks posed by environmental chemicals and there is an urgent need for toxicologists to take a more active role in objective evaluation of the available data published by the scientists, print and electronic media.

In the first group, three chapters discuss advances made in the understanding of antimicrobial and antiviral chemotherapy. In the 1980s, a novel form of gene regulation was discovered in prokaryotes whereby translation of a specific mRNA into protein was suppressed by its hybridization and binding to a counter transcript, i.e. an RNA transcript in opposite orientation to the mRNA. Investigators are now making progress in utilizing this concept of nature in the development of a new class of therapeutic agents called antisense. Early reports from *in vitro* animals as well as human trials are quite exciting. Crook and Bennet describe the progress in the development of antisense oligonucleotides as pharmacological and therapeutic tools. Multidrug resistance represents a major obstacle in the successful chemotherapy of cancer. Studies have demonstrated that P-glycoprotein appears to play an important role in drug resistance. Bellamy in 'P-glycoprotein and multidrug resistance', suggests that one should not over-look other probable mechanisms such as increased detoxification, enhanced ability to repair DNA damage, alterations in cellular metabolism, failure to undergo apoptosis may also be operating. Despite several classes of promising new anti-HIV agents, the clinical emergence of drug resistance variants of HIV has severely limited the long term effectiveness of non-nucleoside drugs. Critical mutations in the RT and PR genes in resistance virus have been identified. Erikson and Burt feel that these insights will have definite implications in design of new drugs and therapeutic strategies to combat drug resistance to AIDS.

In the article 'Molecular mechanisms of toxicant-induced immunosuppression', Hosapple *et al.* describe the role of second messengers in immune regulation and how changes in intracellular signalling by immunotoxic agents (cannabionoids, polyaromatic hydrocarbon (PAHS) etc.) result in immune disfunction.

There are three excellent articles in cardiovascular related area. In 'Angiotensin receptors and their therapeutic implications', Griendling *et al.* describe the current concepts on angiotensin receptor classification, antagonist and their therapeutic utility. At least 30 different phosphodiesterase enzymes have now been identified in mammalian tissues and cells, many of which are products of separate genes. Polson and Strada describe the cardiovascular potentials of various PDE inhibitors. In the article 'Cardiac actions of antihistamines', R. L. Wossley cautions the clinicians about the propensity of some of the new generation antihistamines like terfenadine and astemizole to induce life-threatening arrhythmias and alerts the essentiality of investigating the cardiac toxicity of older antihistamine, which are still under clinical use.

Insulin controls organismal and cellular physiology of numerous intracellular signals. Despite intensive investigations, the basic molecular mechanism of insulin action on glucose metabolism is still elusive. In the review article, 'Insulin signal transduction and the IRS proteins', Myers and White give detailed aspects of molecular mechanisms, in particular the role of IRS protein in insulin action. This is truly an excellent article, well-annotated and of value to anybody working in diabetes research either in the laboratory or in the clinic. Progesterone plays a crucial role in the mammalian reproduction that is: initiation and maintenance of pregnancy. Some of the antiprogestins like mefipristone have strong affinity to both progesterone and glucocorticoid receptors. Spitz *et al.* describe an exciting therapeutic potential of these molecules at low doses for the treatment of endometriosis, uterine myoma, inducing abortion, treatment for steroid-dependent tumours and contraceptive. Along with the impressive body of evidence on the molecular mechanisms of estrogen, Yager and Liehr discuss in 'Molecular mechanisms of estrogen carcinogenesis' the mechanisms of estrogen carcinogenesis with a focus on carcinogenic potentials of estrogen metabolites.

Breast cancer has claimed a unique place in the public consciousness. Over the past decade, and especially within the past few years, breast cancer has become probably the single most discussed medical topic of all – more so even than AIDS and cardiovascular diseases. In recent years we have witnessed a barrage of articles and debates and programmes and information about breast cancer epidemics. The real turning point came in 1990 when scientists finally proved the existence of a gene called BRCA1 which if damaged could predispose women to developing breast cancer. In 'Breast cancer and environmental risk factors', Wolf *et al.* present the epidemiological evidence for breast cancer risk from exposures to chemicals and physical agents in the environment and relevant corollary studies in laboratory animals. Hoyer and Siper, in 'Assessment of follicle destruction in chemical-induced ovarian toxicity' discuss the various aspects of 4-vinyl cyclohexene-induced ovarian toxicity.

There are three excellent articles in the area of molecular mechanisms of action of drugs and chemicals. The first one looks into the role of G-protein molecular mechanisms in signalling process. Results obtained in the past few years have greatly enhanced and modified our understanding of G protein-mediated transmembrane signalling processes. There is a large body of evidence showing that many G protein-coupled receptors do not signal by activating only one G protein to generate one intracellular signal. Possibly more often, different G proteins are activated in one receptor to generate multiple intracellular signals. Gudermann *et al.* explain the G-protein-mediated complex signal network at each transduction level, i.e. receptor, G-protein and effector. In addition to this, Exton in 'Regulation of phosphoinositide phospholipases by hormones, neurotransmitter and other agonists linked to G-proteins' describes the mechanism by which agonists activate phospholipase isozymes. The molecular cloning technique has permitted identification of ten novel isoforms of mammalian adenylyl cyclase, which are membrane bound activated by alpha subunit of G-alpha and by the diterpene forskolin. Sunahar *et al.* review the current understanding of multimodel regulation of adenylyl cyclase system and cAMP synthesis.

There are nine reviews under neuro and behavioral pharmacology. Progressive degeneration of a subset of neurons is the pathologic hallmark of adult-onset neurodegenerative disorders such as Alzheimer's disease, Parkinson's disease and amyotrophic lateral sclerosis. A growing body of evidence points to the involvement of free radicals in mediating neuronal death in these diseases. Simonian and Coyle review advances made in understanding the intracellular pathways involved in free radical generation and how free radicals contribute to selective neuronal degeneration in these disorders. An approach to increase defense mechanisms against free radicals may be an important pharmacological strategy to show or halt neuronal degeneration. Lipid vs protein theory of alcohol action in the nervous system is the subject of one of the most long-standing debate controversies of biology. Once again in this review, People *et al.* discuss both lipid and protein theories of alcohol's action and in addition, its effects on neurotransmitter-gated ion channels are also looked into. Exocytosis is an ubiquitous mechanism for secretion of intercellular chemical messengers. Our understanding of the molecular mechanism is still ambiguous. Augustine *et al.* review a number of perturbation strategies and summarize what this approach has taught us about the functional roles of protein in exocytosis and also molecular model of protein dynamics during exocytosis. Enormous advances have been made toward the understanding of nociception and this definitely modified our approach in discovering future analgesic drugs. Both Dray and Urban feel that new targets including receptors, enzymes transport systems, gene regulation will be the future target for developing antinociceptive agents. Generally opioids exert their pharmacological actions through three opioid receptor classes μ, δ, k, which are identified through molecular cloning technique. Despite this advancement, our knowledge about their involvement in pharmacological actions is not clear. In 'Opioid receptor types and sub-types', Zaki *et al.* describe the concept of multiple δ opioid receptor subtypes which might be helpful in our understanding and development of selective pharmacotherapeutics.

The concept that clonidine, a centrally acting antihypertensive, might be interacting with a receptor recognizing its structure as an imidazoline was proposed by Bousquit *et al.* in 1984. Imidazoline receptors constitute a family of noradrenergic high-affinity binding sites for a clonidine, idazoxan and allied drugs. Although these receptors are not yet cloned, there are abundant biochemical, pharmacological and biological data to indicate that they and α adrenergic receptors are distinct molecular entities. Many questions related to the molecular, cellular, pharmacological and functional relevance of the I receptor family and its endogenous ligands await cloning of the receptor and establishments of the structure of other endogenous ligand. Anabolic androgenic steroids have become more widely used and abused not only by the elite athletes but also by non-athletes for cosmetic purpose as well as promoting aggressive behaviour. Luckas in 'CNS effects and abuse liability of anabolic androgenic steroids', feels that at least the former knows the danger and side effects while the latter tend to be more ignorant of facts. As there are no reliable animal models for characterizing the abuse liability and physical dependence potential of this class of drugs, Lukas feels a better understanding of the CNS effects and abuse liability of anabolic androgenic steroids may come from newly emerging studies of neuroactive steroids and brain imaging technologies. In addition, in 'Pathophysiological basis of vulnerability to drug abuse' Piazza and Moal discuss the role of an interaction between stress, glucocorticoids and dopaminergic neurons. Nicotinic cholinergic systems are believed to be important in memory-enhancing effect and there is a loss of cholinergic innervation from the basal forebrain in pathologies that affect memory, such as Alzheimer disease. The definitive demonstration that nicotine potently enhances transmission in the hippocampus is unquestionably an important step towards recalling nicotine as a powerful modulator of memory. In 'Pharmacology of nicotine', Benovitz describes addictive and therapeutic potentials of nicotine for the treatment of Alzheimer's disease, ulcerative colitis, Parkinson's disease, Tourette's syndrome, sleep apnea, and attention-deficit disorder.

Unusually there are no reviews on allergy, inflammation, respiratory, gastrointestinal, renal pharmacology, pharmacokinetic and natural product topics. It is difficult for editors to cover all topics of medical interest. Nevertheless, the editors have made a valiant attempt to provide the readers with a comprehensive and thorough review of the current topics in pharmacology and toxicology. All chapters in the review are well written, comprehensive to the general and special readers. Thus this annual review provides an important and invaluable source of literature and data.

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The Natural Science of the Human Species: An Introduction to Comparative Behaviour Research – The 'Russian Manuscript' (1944–1948). By Konrad Lorenz. Edited by Agnes von Cranach. Translated by Robert D. Martin. MIT Press 1996, pp.337 US $35.

This book, the first that the Nobel Laureate ethologist Konrad Zacharias Lorenz (1903–1989) wrote, is his last to be published. It was written in the period 1944–1948 while he was a prisoner-of-war in Armenia. The manuscript, called the 'Russian Manuscript', consisted of about 750 pages. As there was no paper available he ironed old cement sacks and used them to write on, improvizing his own ink of potassium permanganate, steel nibs and occasionally bird quills. Apart from scarcity of writing materials he wrote under extremely harrowing circumstances without a desk, poor lighting conditions and freezing temperatures in winter. The book is a tribute to human heroism and perseverance.

This book has much to offer to students of history, sociology of science and to those interested in the development of scientific ideas. There is also something for philosophers in it, even though it must be pointed out that Karl Popper vehemently disagreed with Konrad Lorenz's interpretation of Kant as contained in this book. Its central construct is to give a Darwinian and evolutionary explanation of the philosophical writings of Immanuel Kant. From 1940 until he was drafted into war service, he had briefly shared with Edvard Baumgarten the Professorship of Psychology at the Albertus University of Koenigsberg, a post previously held by Immanuel Kant. The best part of the book is taken up in reconciling the real world and the *a priori* or the phenomenal worlds of Kant. For Kant there were two distinct worlds – the real world outside and the internal world with *a priori* schemata that influence and filter our perceptions. Therefore, for Kant there was no comprehensible and logically tangible relationship between the objective world and the intelligible world, comprising only those *a priori* schemata that create images of objects in our perceptual world. Lorenz is trying to recognize and establish a connection between the two worlds. All *a priori* knowledge consists of evolved genetic representations which are adaptive predispositions that allow humans to make sense of the myriad visual and sensory stimuli originating in the outside world. Those acquainted with Lorenz's work will recognize that he has advocated in all his books and writings the same idea for non-human animals. Thus arose his concepts of 'fixed action patterns' and 'innate releasing mechanisms' which enable animals to instinctively respond to appropriate eliciting stimuli the first time they are confronted with them.

The 'Russian Manuscript' travelled with its author during his entire career, first in Vienna and later as the Director of the Max-Planck-Institutes for Behavioural Physiology in North Germany and in Seewiesen, Bavaria. The manuscript unaccountably disappeared sometime in the 1960s and was discovered by his daughter Agnes von Cranach in 1990 in the family home in Altenberg near Vienna. The wealthy orthopaedic surgeon Professor Adolf Lorenz was a remote and authoritarian father. He thoroughly disapproved of Konrad's adolescent passions (to marry a young woman of his choice, which he later did, and to work with animals). The father was not even impressed with his first landmark paper on the behaviour of a tame jackdaw and openly said that the only good bird was one on the table ready to be eaten!

The real question is, what scientific value could a book written fifty years ago have today. This book cannot even be viewed as a zeitgeistdokument since it does not contain contextual references. Much of the ideas and material found here have found their way into books that Lorenz later wrote *On Aggression, Die Rückseite des Spiegels* and his textbook *The Foundations of Ethology*. In fairness Lorenz did not want to publish this book in the form it then had (and apparently still has today) nor had he decided on a definitive title. The present title is quaint and does not give even an inkling about its rather rambling contents. It does appear, however, that Lorenz had already conceived in his mind the many and varied conceptual contributions he would be making over the next three decades. This in itself is a fascinating phenomenon. But then Lorenz's working methods were most unusual even for an ethologist. John R. Krebs had this to say of his empirical research style: 'He rarely did replicated, controlled experiments, but reached conclusions, sometimes correct and sometimes, with the benefit of hindsight, probably incorrect, on the basis of careful and detailed observation of the spontaneous behaviour of animals living around him'. An unwitting but apt comment was made many years later at Seewiesen, by F. Schutz, who once asked Konrad during a seminar: 'Is that something that actually happens or is it just something you saw?'.

It is very unlikely that the book under review will be anywhere as popular as some of his earlier charming books such as *King Solomon's Ring* and *Man Meets Dog*, even though the writing style is already quintessential Lorenz (samples: p. 19, Kants premise that there is no relationship between these two worlds is incorrect. p.20, The infinite quest for knowledge that drives the natural sciences fundamentally prevents us from making any voluntary concessions to agnostics. We simply do not believe that there is anything in the universe that is fundamentally unknowable. p. 187, It was Kant who coined the delightful sentence: there are two things that never-endingly fill the human spirit with wonder – the starry sky above and the moral law within. p. 247... Heinroth habitually referred to philosophy as the pathological idling of innate human functions intended for an understanding of nature etc).

Lorenz is preoccupied in several chapters on vitalism, mechanism and their implication for comparative behaviour research (ethology). Fortunately for biology these isms and schisms have been subsequently excoriated by the scientific insights made by developmental biology, genetics and molecular biology. There is delightful chapter written with much feeling on 'Animal keeping as a research method'. Even his stern parents had allowed him as a boy to maintain at home a bestiary which included invertebrates from the Danube, tropical fish species, amphibians and reptiles, hand-raised birds, night herons, ravens, little Grebes, and an eagle. Not surprisingly much of his later researches was based on observing small groups of tame and semi-tame animals living in his house or laboratory. Those who have known the life and career of Konrad Lorenz and have been drawn to his work and have been influenced by it will find this unusual book most invaluable.

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Joseph Needham: 20th Century Renaissance Man. Profile. By Maurice Goldsmith, UNESCO Publishing. 1996.

Joseph Needham was an unusually talented 'polymath' scientist who combined scientific curiosity with Christian humanism. He was gifted with poetic romanticism mingled with political radicalism. In the first half of the 20th century, Noel Joseph Terence Montgomery Needham belonged to the radical intellectual movement and was part of an 'invisible college'.

President and master of Gonville and Caius College, Cambridge, and OM, FRS, and FBA, Needham was the recipient of innumerable national and international honours. It is of historical interest to know that he was also responsible for the inclusion of 'S' for Science in UNESCO.

And at the end of the World War II, when Julian Huxley was invited to organize the UNESCO, he asked Needham to assist him in the gigantic task of planning and executing the first global organization of its kind under the Charter of the United Nations. Needham was the author of many scientific works – books and articles – concerning morphology and biochemistry that created synergism of science and religion, and of political activism with academic social responsibility. In his search for scientific universalism he became the historian of Chinese science and technology. He produced and edited voluminous investigative and interpretative studies of scientific principles belonging to the mysterious land of the Orient.

But Needham was also a man of spiritual commitment who endeavoured to create the Kingdom of God on this Earth and in his entire awakened years (1900–1995) he loved divinity in humankind beyond consideration of nation, race, gender and religion. He was, however, a Christian by faith but his respect for religion was more philosophical and less theological. He was a regular visitor to the Thaxted Church where he often delivered Sermons. Topics of his lecture from the pulpit included Ethics of Economics and Science, Against 'Holy War', Ideas of the 'Just War', Religion and Superstition, and Shintoism, Japanese Confucianism and the Cosmic Christ. He spoke about the 'Place of Women in the Church' long before we learnt about the LIB and political correctness. Needham was also concerned with the eternal struggle of poverty and often spoke about 'Greed and Capitalism'. He spoke about the need to create an 'International Language and Science'. He worked to liberate Christianity from its age-old blind faith and make the Church and Faith the vector of Scientific Knowledge (Truth). But to achieve a modern scientific civilization, Needham recognized social justice as the prime condition and for this he committed himself and his colleagues to social responsibility.

Maurice Goldsmith has described Needham as 'the bridge builder', and truly Jospeh as a scientist observed similarities and dissimilarities and tried to perceive Unity in Diversities. He recognized many interested scientific principles of chemistry and physics in the ancient writings of China. And even if we cannot say why the technological–industrial revolution took place in Europe in the 17th century, there is no scientific reason to believe that any race or people could be devoid of scientific acumen. The historical studies of Chinese science discovered, translated and published by Joseph Needham contributed immensely in creating awareness about the historical contribution to world civilization by China.

In search of scientific universality Needham travelled across the oceans and flew over the fighting forces of East and West, and drudged through the marshes and backwaters of China. He crossed the traditional racial and linguistics borders to befriend with his Chinese colleagues and students; and learned the language of distant people and after the death of his first wife Dorothy, eventually married the Chinese scientist, Dr Lu Gwei-Djen, in 1989, who became Needham's collaborator–editor of the voluminous *History of Chinese Science, Civilization and Society*.

The book provides interesting details of this historic association of the aristocratic-turned Christian socialist scientist and his scientific collaborative work with the Chinese scientist, Gwei-Djen whom Needham came to know for the first time in 1937, when the young Chinese student worked under Dr (Mrs) Dorothy Needham. In the pursuit of science, the three were united as in a common cause. The three were attracted to each other with scientific-spiritual devotion without causing acrimonous damage to each other or to scientific research. Bound by love and respect for each other they proved the model of social harmony. Joseph had remained married to Dorothy for sixty-three years (1924–87), but his second wife died within two years of their marriage.

The book under review describes the foundational influence of Joseph's bourgeois class-conscious English father who was a medicalman by profession. During his younger years, Joseph often attended surgical operation theatre where the senior Needham was an anaesthetist at large military hospitals in London. But surgery held no lasting intellectual interest for Joseph Needham. The author describes his father's influence in some detail but in this age of politically correctness he has ignored the role of his mother. In fact the two main female characters – Needham's mother and his first wife – receive no due consideration in this otherwise extremely interesting and informative study.

This Profile of Needham volume contains a chapter entitled 'Egg and Embryo' in which the author discusses Needham's scientific contribution to pure research in early embryology. In the 1930s, he was seriously involved in developing the technique of embryological micro-operations in the laboratory of Albert Brachet in Brussels. Along with Waddington and his first wife Dorothy, he worked at the Institute of Otto Mangold in Berlin, and the research done during this period was from a theoretical point of view of historical significance for regions of biology even outside embryology, wherever stimulus–response reactions were concerned.

In *Biochemistry and Morphogenesis*, (published in 1942) Needham, Waddington and others explained that a non-living substance can act as an organizer for the embryonic differentiation of a nervous system. Needham in the study claimed that a piece of boiled mouse heart placed among the living cells of a human embryo induced the formation of a secondary or 'extra' brain. This was evidently of philosophical significance, as it demonstrated that there was a physico-chemical explanation for what had been for centuries believed to be the work of an all-knowing Divinity. (page 40).

Needham's associations with eminent Church leaders influenced him in his scientific-philosophical orientation, and his research findings made him realize that while natural sciences themselves were wonderful achievement of human mind, study of the past achievements of ancient civilizations is also of significance for history of science developed through the ages, and even if from small beginnings the contribution of the ancient people to science is not of less importance.

Julian Huxley commenting on Needham's embryonic research stated that this marks another milestone on the road that biology was taking in its transformation from a statistic to a dynamic science. 'Biologists are under a very real debt to Dr Needham for his wide reading, his critical and constructive facility, and his patient industry which has issued in his fine work'. (*Nature*, 6 February 1932, quoted by Goldsmith, page 39).

The author of this Profile of the 20th Century Renaissance Man, is the master craftman of the scientific Profile of another Renaissance Man – J. D. Bernal. But, then, Maurice Goldsmith himself is a Renaissance Man who at the end of World War-II poineered the study of Science and Public Policies, and introduced the concepts of Popularization of Science and the Science Critics. Closely involved in the development of UNESCO, Goldsmith was appropriately equipped to prepare this Profile of Needham which reads like a poet even though it is full of information of academic interest and historical importance. Needham's China visit in search of scientific classics provides fascinating reading, but more significant are the details of his journey through the backward countryside of China, and his meetings with his Chinese hosts who were exemplary of cooperation with scientific information and warm oriental hospitality.

Maurice Goldsmith's treatment of his Needham Profile is unusually distinct as it is attractively unconventional. The volume opens with an address to Joseph Needham from the author. But it has to end in Obituary to Needham, in P.S.:

> 'Since I wrote the above, I have experienced the sadness of your death on the evening of Friday, 24 March 1995. What has intrigued me is the extensive space which the broadsheet dailies, in particular, have devoted to your obituary...'.

The *Profile* of Joseph Needham is a masterpiece work on a life of the colossal man of science and philosophy in whom were united conflicting forces of Faith and Reason, and whose life offered synergism of knowledge and action. He commanded love and affection and high honour of the academic establishment.

Even though to avoid confrontation with conservative establishment of the Royal Society, Needham used a pseudoname when writing radical political articles, his contribution to contemporary critical science movements has been most appropriately placed in historical perspective by Maurice Goldsmith. The author deserves felicitations for this valuable gift to general readers and to the academic and scientific community at large.

DHIRENDRA SHARMA

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MEETINGS/SYMPOSIA/SEMINARS

STORMCUP '97 (Short Training on Research in Marine Cyanobacterial utilization Programme – 1997)

Date: 10–21 February 1997
Place: Tiruchirapalli

The training provides hands on experience on various techniques using different instruments such as LS Counter, GC, HPLC, Electrophoresis, Photomicrography and ELISA. Lectures and field trip to Gulf of Mannar area for field experiments and collection are also arranged.

Contact:
Dr G. Subramanian, Director
National Facility for Marine Cyanobacteria
Bharathidasan University
Tiruchirapalli 620 024
Phone: 0431–660352
Fax: 0431–660384
E-mail: nfmc@bdu.rect.ernet.in.

A Short Term Course on Soft Condensed Matter

Date: 10–15 March 1997
Place: Vadodara

The course will consist of series of lectures with a few tutorials, practicals and will cover the following topics: Surfactants and micro emulsions, Polymers, Colloids and biocolloids, Ferrofluids, Surfaces and interfaces. Experimental methods include: Microscopic techniques (rheology, conductivity, etc.), Scattering techniques (SANS, SAXS, Light scattering), Electron microscopy, Relaxation techniques (NMR, ESR, etc.), Computer simulation. Interested Ph D students and young researchers may apply.

Contact:
Dr P. S. Goyal
Solid State Physics Division
Bhabha Atomic Research Centre
Trombay, Mumbai 400 085
Phone: 022–5563060 Extn. 4236
Fax: 022–5560750
E-mail: psgoyal@magnum.barc.ernet.in.

National Symposium-cum-Workshop on Leptospirosis

Date: 11–13 September 1997
Place: Port Blair

Contact:
Professor S. C. Sehgal
Chairman or Dr V. G. Rao, Organizing Secretary
Regional Medical Research Centre
Port Blair 744 101
Phone: 91–3192–21156, 21104
Fax: 3192–30175
E-mail: icmrrcpb@ren.nic.in.

INDIAN ASSOCIATION FOR THE CULTIVATION OF SCIENCE
JADAVPUR, CALCUTTA 700 032

IACS/ADVT/P/4/96

Indian Association for the Cultivation of Science is looking for creative scientists for appointment at the level of:

(1) **Reader** (one post) in the Polymer Science Unit in the scale of Rs 3700–125–4950–150–5700.

(2) **Lecturer** (one post) in the Spectroscopy Department in the scale of Rs 2200–75–2800–EB–100–4000.

(3) **Lecturers** (two posts) in the Solid State Physics Department (in the above scale). Persons having good academic record and capable of initiating and guiding research in the frontier areas of

- (i) Polymer Science
- (ii) Experimental or Theoretical Spectroscopy
- (iii) Experimental Solid State Physics, respectively.

N. B.: One post of Lecturer in Solid State Physics Department is reserved for SC.

Applicants are requested to send their Bio-data along with list of publications, names of referees, expected minimum salary, etc. (six copies each) to the Registrar, Indian Association for the Cultivation of Science, Jadavpur, Calcutta 700 032 before 7th February 1997.

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The posts carry allowances as are admissible to the Central Government Employees stationed at Calcutta. The total emoluments at the initial of the Scale will be Rs 9943 and Rs 6888 for Reader and Lecturer respectively.

(P. C. GHOSH)

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
(CSIR), NEW DELHI, INDIA

ADVERTISEMENT NO. 15/96

Position: Director, National Physical Laboratory (NPL), New Delhi.

CSIR, the premier agency established by the Govt of India to undertake scientific and industrial research in the country, is looking for suitable scientist/technologist for the post of Director for its National Physical Laboratory (NPL), New Delhi. NPL, Delhi is a multidisciplinary laboratory and is devoted to carrying out R&D work to advance the frontiers of knowledge in various areas of Physics and applying the knowledge for the technological advancement of the country; to establish, maintain and improve national standards of measurements and to realize the 'Units' based on International System; to assist industries, national and other agencies by offering consultancy services for precision measurements, calibration, development of devices, processes, and solving other allied problems related to Physics. The major R&D programmes of NPL are (i) measurements, standards and calibration; (ii) material characterization; (iii) materials development including carbon–carbon composites, piezoelectric ceramics and ceramic–polymer composite materials, thin film optical coatings and systems, opto-electronic materials and devices, etc., (iv) radio and atmospheric sciences; (v) cryogenics and super-conductivity, etc. The Laboratory offers consultancy services to industry, R&D units, academic institutions and government agencies in the areas of its specialization.

The post carries the pay scale of Rs 5900–200–7300 plus allowances as admissible to Council officials. The appointment to the post will be on contract for a period of six years or till superannuation, whichever is earlier.

For further details, please contact the Director-General, CSIR, Rafi Marg, New Delhi 110 001 (India), (Fax, 3710618; Telex, 031–65202 CSIRIN, 031–66147 CSIRIN; Gram, CONSEARCH, New Delhi).

Interested candidates may send their complete biodata by **25 February 1997** to the Director-General, CSIR, Rafi Marg, New Delhi 110 001.

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH
(CSIR), NEW DELHI, INDIA

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Position: Director, Regional Research Laboratory, Jorhat.

CSIR, the premier agency established by the Govt of India to undertake scientific and industrial research in the country, is looking for suitable scientist/technologist for the post of Director for its Regional Research Laboratory (RRL), Jorhat. The Laboratory is devoted to developing indigenous technologies based on the natural resources of the North-Eastern region and to help in the economic development and industrialization of the North-Eastern region in particular and the country in general. The major R&D programmes of the Laboratory relate to development of oil-field chemicals, agrochemicals, agrotechnologies for aromatic and medicinal plants, drugs and drug intermediates, inorganic chemicals, speciality papers and boards and building materials. In addition, basic research in the areas of Organic Chemistry and Bio-chemistry and Geosciences is also carried out. The Laboratory has one branch Laboratory at Itanagar and field stations in Mokok Chung (Nagaland) and Imphal (Manipur).

The post carries the pay scale of Rs 5900–200–7300 plus allowances as admissible to Central Govt officials. The appointment to the post will be on contract for a period of six years or superannuation, whichever is earlier.

For further details, please contact the Director-General, CSIR, Rafi Marg, New Delhi 110 001 (Fax, 3710618; Telex, 031–65202 CSIR IN, 031–66147 CSIR IN; Gram, CONSEARCH, New Delhi).

Interested candidates may send their complete biodata by **31 January 1997** to the Director-General, CSIR, Rafi Marg, New Delhi 110 001.

WADIA INSTITUTE OF HIMALAYAN GEOLOGY

33, General Mahadeo Singh Road
DEHRA DUN 248 001

Contact Programme on Field Studies of Deformation Tectonics for Young Scientists

Department of Science & Technology, Govt of India has been organizing Contact Programme for Young Scientists over the past several years, with the idea to bring young scientists in contact with eminent scientists in relevant fields, train them in frontline areas, and expose them to major national laboratories and field studies. The next programme in Earth Sciences is planned at Wadia Institute of Himalayan Geology, Dehra Dun from 21 March 1997 to 30 April 1997. The planned programme will focus on field studies of structure metamorphism and neotectonics. Applications for participation in the Programme are invited from candidates who have just completed M Sc (Geology) or who have just started their M Phil/Ph D (Geology). The selected candidates will be paid second class train fare and reservation charges from their place of residence to Dehra Dun and back.

Apply on a plain paper affixing a recent passport size photograph and mentioning the following:

1. Name
2. Date of birth
3. Academic record (starting from Inter/HSC) with year, Percentage of marks obtained, Board/University
4. Present status
5. Any other matter in support of your candidature.

The applications addressed to Dr M. I. Bhat, Wadia Institute of Himalayan Geology, Dehra Dun must reach on or before 31 January 1997.

Nominations are invited for

WINTER SCHOOL ON 'ATOMIC FORCE MICROSCOPY'

Sponsored by
Department of Science & Technology
Govt of India, New Delhi
Under Science & Engineering Research Council Scheme for Young Scientists (SERCYS)

Objective : To expose younger scientists through lectures and demonstrations with latest techniques of force microscopy for imaging of technological/biological surfaces

Time Schedule : 13 February 1997 to 18 February 1997

Participants : A maximum of twenty (20) participants selected out of the nominated candidates from various National Labs/Universities, IITs, RSICs, etc.

Organizing Institute : Central Scientific Instruments Organization, Chandigarh

Venue : Central Scientific Instruments Organization, Sector 30, Chandigarh 160 020

Eligibility

Age : Less than 35 years

Academic Qualifications : Minimum M Sc/M Tech (Please attach a copy of the Biodata)

Nature of work/job : Engaged in R&D/characterization of technological/biological surfaces. (one page write-up concerning nature of work being done by the participants should accompany the nomination)

Assistance from Organizer/ Sponsors : Travel charges as per DST rules will be reimbursed.
Free boarding/lodging

Last date for receiving the nominations : 25 January 1997

Contact/correspondence address:

Dr S. S. Wadhwa
Head, Micropositioning Systems Division,
Central Scientific Instruments Organization,
Sector 30, Chandigarh 160 020
Phone: 0172–657449; Fax: 0172–657082/657267.

MINISTRY OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF SCIENCE AND TECHNOLOGY

JSPS POSTDOCTORAL FELLOWSHIPS: 1997–98

Department of Science and Technology (DST) invites applications from promising and qualified researchers for Postdoctoral Fellowships to Japan for 1997–98 offered by the Japan Society for the Promotion of Science (JSPS) under its JSPS Postdoctoral Fellowships for Foreign Researchers Programme. There are five fellowships to be nominated by DST under the framework of India–Japan Cooperative Science Programme (IJCSP).

Priority areas in which applications are invited

1. Molecular Structure, Spectroscopy & Dynamics
2. New Materials
3. Manufacturing Sciences
4. Modern Biology & Biotechnology, and
5. Astronomy & Astrophysics.

Eligibility

Applicant should:

1. be a citizen of India;
2. be an affiliate of a recognized scientific institution in India including researchers like CSIR, UGC JRFs, SRFs and RAs;
3. hold a doctorate degree when the fellowship goes into effect, which must have been received within six years prior to April 1, 1997;
4. have arranged in advance his/her research plans with his/her Japanese host researcher; and
5. be able to forward the application through proper channel of the parent institution.

Age: Preferably below 45 years.

Duration and commencement of Fellowship

1. Fellowships are awarded for a period of 24 months (if one does not require 24 months to complete the research in Japan, one may propose a shorter fellowship tenure of at least 12 months);
2. Each Fellowship must begin between April 1, 1997 and March 31, 1998.

Host research institutions in Japan

Fellows can conduct their research in the following Japanese institutions:

1. Universities;
2. Inter-university research institutes;
3. Research institutes/scientific research corporations under the jurisdiction of the ministry of Education, Science, Sports and Culture (the Monbusho).

Terms of awards

JSPS provides each Fellow with the following financial support:

1. Round-trip air ticket for the Fellow only;
2. Monthly stipend of Y270,000;
3. Settling-in allowance of Y200,000;
4. Monthly housing allowance not exceeding Y100,000;
5. Monthly family allowance of Y50,000 if accompanied by dependents;
6. Accident and sickness insurance coverage for the Fellow only.

In addition, application may also be made for the Monbusho's Grant-in-Aid for JSPS Fellows (Tokubestu Kenkyuin Shorei-hi) of up to a maximum of Y1,500,000 per year to cover cooperative research expenses such as for equipment, materials, supplies and domestic travel. Grant applications must be prepared by your host researcher and submitted through your host institution.

Application procedure

The Application Forms can be requested from the Programme Office, India–Japan Cooperative Science Programme (IJCSP), International Division, Department of Science & Technology, Technology Bhavan, New Mehrauli Road, New Delhi 110 016. Request for application should be received **latest by February 15, 1997 along with a self-addressed unstamped envelope (12 cm × 27 cm) entitled 'JSPS PD Fellowship FY 1997'.**

The duly filled in application form should be forwarded through proper channel **latest by March 15, 1997.**

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A fortnightly journal of research

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GOVERNMENT OF INDIA

DEPARTMENT OF SCIENCE & TECHNOLOGY

Programme on Nonlinear Phenomena, Complex Matter and Biologically Inspired Physics

The importance of nonlinear science, soft condensed matter (such as colloids, powders, polymers and membranes) and biologically inspired physics has emerged very clearly in recent years. In view of this and given the large mutual overlaps of these disciplines as well as their interdisciplinary nature, the DST has considered it fit to constitute a separate Programme Advisory Committee (PAC) specifically to foster research in these areas. Suitable project proposals are invited in the above general fields.

Research proposals should pay particular attention to the development and application of ideas and techniques that cut across disciplines and/or seek to elucidate and provide insight into the general paradigms of complexity and nonlinearity.

Proposals that come under this general purview include:

Dynamical phenomena far from equilibrium, driven systems, chaos, especially spatiotemporal. This encompasses both model systems such as sandpiles and driven lattice gases as well as 'real' examples such as colloids in shear flow and sedimentation, turbulence, kinetics of phase ordering, granular matter (powders), earthquakes, pattern formation, etc.

Physics of soft condensed matter. Membranes (biological and model), Langmuir monolayers and Langmuir–Blodgett films, micelles, vesicles, lamellar structures, microemulsions, emulsions, foams, and other structures in surfactant solutions: liquid crystals, both thermotropic and lyotropic, polymer solutions, gels, and melts: colloidal suspensions, including ferrofluids, electro-rheological fluids, etc.

Biologically inspired physics. Models in evolutionary biology and population genetics, evolution of complexity, neural networks, optimization, protein folding, motor proteins and molecular ratchets, etc.

Qualified researchers interested in submitting project proposals to be considered for funding may write to the following address for further details:

The Convener (Dr Praveer Asthana)
PAC on Nonlinear, Complex and Biological Systems
SERC Division
Department of Science & Technology
Technology Bhavan
New Mehrauli Road
New Delhi 110 016

Founded: 1932

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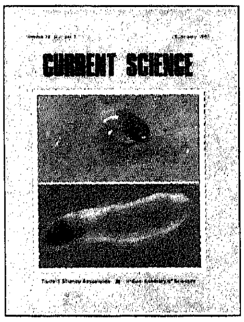

COVER. The migratory slug-shaped 'embryonic' stage in the life cycle of the social amoeba *Dictyostelium discoideum.* The amoebae making up the slug in the top panel were stained with neutral red, a dye specific for pre-stalk cells. The slug in the lower panel consists of amoebae transformed with the *E. coli* beta-galactosidase gene under the control of a pre-stalk-specific promoter. Both slugs are about 1 mm long. The problem addressed in the paper by Atzmony *et al.* (see page 142) is: why do some ameobae adopt the apparently altruistic pre-stalk pathway? (Pictures: courtesy M. Azhar)

Indexed in CURRENT CONTENTS/GEOBASE/CHEMICAL ABSTRACTS

In this issue

Fullerenes

There have been few discoveries in recent times that have had as much of an impact on chemistry as the discovery of the fullerenes. Ever since C_{60}, buckminsterfullerene (named after the father of the geodesic dome, Buckminster Fuller) was conveniently synthesized, by laser ablation of graphite, the literature of chemistry has been flooded with innumerable investigations of new forms of carbon. The inevitable happened in 1996, when the impact of the fullerenes was finally felt in Stockholm with the award of the Nobel Prize in chemistry to Kroto, Smalley and Curl, the three original discoverers of C_{60}. Pradeep (**page 124**) presents an overview of recent research on these new carbon forms, which have stimulated thousands of chemists all over the world. Despite much initial promise in diverse areas ranging from superconductivity to near pharmaceuticals, the fullerenes are primarily still a laboratory curiosity.

In retrospect, the surge of interest in the fullerenes was really fuelled by the remarkable beauty and simplicity of the C_{60} structure and the realization that there must be a treasure trove of all carbon molecules to be discovered. The idea that there could be several new carbon allotropes led to a 'gold rush' resulting in an amazing flood of publications.

The fullerenes brought to chemistry the same frenzied race for new discoveries that was witnessed in physics, following the discovery of high T_c superconducting materials. To the historians of science, superconductivity and fullerenes will provide fascinating case studies, when an analysis is conducted of the state of chemistry and physics in the final decades of the twentieth century.

P. Balaram

Suicide in amoebae – altruism or selfishness?

When a honeybee colony is disturbed, worker bees will unhesitatingly attack the marauder with a painful sting, notwithstanding the fact that for the bee, to sting means to commit suicide. The sting of the honeybee is armed with barbs pointing inwards so that when firmly lodged into the skin of the victim, it cannot be withdrawn. When the bee flies away, its abdomen will rupture and the sting, the poison gland and a portion of the bee's intestines will be left behind. This ensures that although the bee dies in a few minutes, the almost invisible poison gland will keep pumping venom into the victim's body for considerably longer than the bee might have been able to do. When free-living amoebae of the cellular slime mold *Dictyostelium discoideum* exhaust their local food supply, they will come together and form a multicellular slug which will differentiate into a stalk made of dead cells and a spore filled with live cells. The stalk helps the spore rise above the ground and thus increase its chances of being carried away to a new, hopefully nutrient-rich habitat. None of the amoebae are individually up to this task of dispersal but by the sacrifice of some of them, others have gained a finite chance of survival. Despite the extremely different proximate mechanisms by which such altruistic behaviour may be achieved in the bees and the amoebae, there is an underlying similarity in the evolutionary consequences of their behaviour – some individuals behave in a manner that increases the Darwanian fitness of others while decreasing their own fitness.

Providing a common interpretation for the behaviour of amoebae and bees is a triumph of modern evolutionary biology but the mechanism by which such behaviours are shaped by natural selection remains a matter of much debate. Prior to the 1960s, the standard explanation was that any behaviour that is good for the group as a whole would be favoured by natural selection even if it is bad for the individual performing it, because natural selection was thought to always act at the level of the group and override the interests of the individual for the sake of the group. Although this interpretation found much favour in Victorian England, the recognition of the susceptibility of such group selection to invasion by cheaters led to the development of the theory of kin selection, a theory which argued that altruism towards genetic relatives is more likely to evolve as the sacrifice by the altruist can be compensated by the reproductive success of the recipient. Amotz Zahavi, who sees in the arguments of kin selection, the same instability due to invasion by cheaters that others see in group selection, has achieved the status of a lone crusader for the lost cause of good old individual selection. According to Zahavi, there is really no altruism – all apparently altruistic acts are a veiled form of selfishness as individuals attempt to do the best they can under the circumstances they find themselves in. In this crusade, Zahavi is literally moving from place to place and from organism to organism, enlisting the cooperation (altruism?) of the not too skeptical, to push individual selection arguments to their logical conclusion.

On **page 142** of this issue we see the result of this stop-over at Bangalore and at the cellular slime molds, as Daniella Atzmony, Amotz Zahavi and Vidyanand Nanjundiah attempt to explain the apparently altruistic behaviour of the amoebae on the basis of individual selection. According to their bold hypothesis, high quality amoebae produce a poison and coerce low quality amoebae to accept an altruistic role, the alternative for the latter being even more certain death. Whether or not individual selection will eventually be adjudged as a satisfactory explanation for altruism in the slime molds and other organisms, an honest attempt to examine the possible role of individual selection is extremely valuable, and will prove to have been so even if the final answer is in the negative. And the biochemical implications of the concepts they introduce – quality, poison and coercion – are bound to provide new directions for more empirically minded investigators.

Raghavendra Gadagkar

Current Science

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EDITORIAL

Reaching out to Industry

The wide and alert readership of *Current Science* and the support of a few government departments and agencies, have contributed in making *Current Science* an active and speedy medium of communication among scientists in India. *Current Science* is also shaping to be an important forum for information and debate on policy matters relating to science and technology (S&T). In the first three decades of its existence, this journal published 5 to 6 pages of news, containing accounts of new processes developed by industries, patents, articles on technology, engineering development and applied science. It is now all the more necessary to recapture that vision with greater vigour. There is a general belief in the Nehruvian statement that the future belongs to those who make friends with science. It is also being increasingly realized that the future of science in India will be healthier and brighter if the S&T community interacts more closely with users and industries.

We need not repeat here the successes of industry–science interactions, which have made many Western countries (and companies) great economic powers. Closer to home Japan and several newly industrializing countries have demonstrated the role of this nexus. In India too, we are not without several such examples although missed opportunities far outnumber our success stories. Clearly, the time is ripe to jettison our inhibitions and approach the issue of industry–academia interactions with fresh vigour. *Current Science* can provide a valuable forum for developing a new synergism between academic science and technological innovation. This journal would like to invite participation from a wide cross-section of R&D laboratories in promoting both basic and applied science. We look forward to interactions with scientists and technologists in private laboratories, as readers, authors, referees and critics.

Although the columns of this journal have largely catered to academic researchers in India, over the past few years the scope and reach of the journal has been considerably enhanced. In keeping with the times this seems an appropriate moment to reach out to industry. Science in India has rarely been driven by technological compulsions, with research often remaining an esoteric activity. However, with imagination and drive there is every likelihood that a new and effective partnership can be forged between industry and academia, which will eventually enrich the Indian scientific scene.

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Undermanaging health care: Lessons of the plague

Current Science should be congratulated for publishing a special section on the 'Plague epidemic of 1994' (*Curr. Sci.*, 1996, **71**, 781). The report sets at rest once and for all, the controversy regarding the etiology of epidemics between August and October 1994 in the Beed District of Maharashtra and the Surat city of the state of Gujarat. The Technical Advisory Committee (TAC) on plague deserves our gratitude for completing so thoroughly the task assigned to it.

The report, however, highlights some glaring deficiences not only in our health care delivery and surveillance systems, but also in our administrative machinery. Unfortunately the scientific community does not emerge out in flying colours either.

TAC was able to bring together the scientific expertise – from epidemiology to molecular biology, existing within the country, to unravel the total story of the epidemic. The unedifying controversy amongst the various groups of 'investigators' at the peak of the epidemic, not only created confusion amongst the people but also discredited the scientific community nationally and internationally. Notwithstanding Ramalingaswami's words of praise for the Government in dealing with the situation, it is obvious that the existing scientific capabilities were neither mobilized nor co-ordinated with any sense of emergency or responsibility. The whole episode gives credence to the adage, 'There are no underdeveloped countries, only undermanaged ones.'

To start with, I must give all credit to the multiple purpose worker who initially suspected the diagnosis and alerted the medical officer at the Primary Health Centre. This is even more creditable considering that the disease was considered to have disappeared from the country – no proven cases having been reported since 1967. The blame for creating confusion must, therefore, rest with so-called 'specialists' and health administrators. It is unfortunate that those with real expertise, and not just 'assumed competence', were not involved at the very outset. As a matter of fact, it is regrettable that at least some of the competent persons who in the very beginning offered their services voluntarily, were denied access to the available pathological material and even ordered to surrender whatever they had. I make this statement with full sense of responsibility, having personally talked to the then Director General, Health Services, Government of India on this issue at that time. His assurance to remedy the situation obviously did not materialize. There was inadequate and unsatisfactory collection of specimens throughout the fortunately short-lived epidemic. This assumption is confirmed by the TAC report (*Curr. Sci.*, 1996, **71**, 783), 'The serological investigations in Beed and Surat outbreaks were handicapped by lack of availability of paired serums at appropriate intervals. Acute phase sera were generally not available.' In addition, one wonders as to who was at fault for not obtaining aspirates from bubos for establishing the diagnosis of the Beed outbreak. This is not the first time that such a glaring lapse has occurred. Some years ago during an outbreak of gastroenteritis in Delhi, there was enough confusion about whether it was an epidemic of cholera. Enquiries soon after from the then Director General, Indian Council of Medical Research whether efforts had been made to collect and preserve appropriate blood and stool specimens for detailed studies failed to elicit the necessary information. Obviously these were not.

The report also highlights the fact that the agency/agencies primarily responsible for surveillance and investigations of such epidemics are either ill-equipped or ill-prepared to meet the challenges of the occasion. The Defence Research and Development Establishment (DRDE) which ultimately undertook the task of isolation and characterization of *Y. pestis* from rather unsatisfactory stored cultures, needs to be congratulated. However, one presumes that the agency primarily responsible for it was unable to discharge the responsibility satisfactorily! It is regrettable that scientists at NICD – the National Centre for Plague – are in need of strengthening 'in its microbiological and molecular genetic capabilities' (page 786). Obviously this weakness was not recognized early enough to mobilize from the beginning scientists at AIIMS, PGI, DRDE, CCMB, who finally were able to establish the cause of the epidemic utilizing the latest diagnostic techniques. I am tempted to quote from the book review on *Plagues. Their Origin, History and Future* in the same issue of *Current Science* (page 807), 'It is one of nature's supreme ironies that, during the unusual juxtaposition of circumstances that leads to an outbreak of plague, all the major participants in the drama are sick. This includes the humans, the rats, the fleas and the very bacteria themselves.' To this may be added the national health administration!

Willis in his book attributes the low mortality associated with the 1994 plague to 'band aids' (antibiotics and insecticides) but cautioned about the need to address the underlying reasons that provoked the epidemic in the first intance. Panda *et al.* who carried out the molecular characterization (see pages 794 –799) observed the unique ribotype of the *Yersinia* from Beed and Surat, quite different from isolates tested from other regions of the world. One wonders if after the completion of its assigned task TAC has been able to create a working group to undertake further studies and ensure continued surveillance.

It is gratifying to note that in April 1996, nearly two years after the event, the Government of India has established a National Apical Advisory Committee (NACC) for National Disease Surveillance and Response System. Obviously believing in the infallibility of our Administrative Services, the Committee is to be headed by the Secretary of Health, GOI and not the Director General of Health Services or an eminent biomedical scientist.

The recent episode of dengue fever, with a high incidence of haemorrhagic and shock syndrome cases, predicted by medical scientists in the country well in advance (Lall, R. and Dhanda, V., *Natl. Med. J. India*, 1996, **9**, 20) once again raises serious doubts about the national will to meet the challenges of emerging and reemerging infections notwithstanding the creation of NACC. For successful implementation of the tasks assigned to NACC require the establishment of a network of surveillance centres distributed throughout the country, staffed with properly trained epidemiologists, microbiologists and molecular biologists who unfortunately are in very short supply. In any case it is good that at least the need for such a task force has been realized.

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New research board for Navy-oriented ocean sciences

The Defence Research and Development Organization (DRDO) has set up a Naval Research Board, effective September 1996. Modelled after the Aeronautics Research & Development Board (AR&DB), the Naval Research Board (NRB) has been set up to strengthen and deepen the knowledge base applicable to the Indian Navy. The NRB has several new features and flexibilities in concept, funding philosophy and operational latitude, elaborated in the Concept Paper, Charter and Executive and Financial Powers of the NRB. Says the 'Concept Paper' in extract:

'If India is to build up the required sea-denial/sea-control capability foreseeable up to 2010–2015 then it will call for appropriate provisioning of a Navy of approximately twice the present size with new weapon systems, surveillance and EW capabilities in all three dimensions. But doing so by import will present insurmountable difficulties; for: *what is developed abroad will not suit our new requirements; what is suitable will be denied; what is not denied will be un-affordable*. This is the already recognizable triple-trap that the Navy will enter in the early years of 2000+.'

Basic philosophy articulating the NRB

The Naval Research Board (NRB) will focus on the generation or enhancement of the applicable knowledge base leading to the exploitation of the sea for military purposes. 'It is important to recognize that the basic philosophy articulating the NRB is the enhancement and expansion of the applicable knowledge base. It is not the definition or achievement of a future naval operational requirement. The aim is increasing our understanding of a topic area. The NRB's projects will be imagination-driven – not requirements driven.

'The NRB will invite research proposals that focus on substantial phenomena or observations that are not understood, and, that lack of understanding is a serious obstacle to scientific or technological progress.' Elaborating:

- The search is for bright people with novel and/or imaginative approaches. 'If you had all the resources you need to do what you want to do, what would you be doing that you are not doing now.' The unit of funding is the experienced leader–researcher and his small team ~ typically one post-doctoral researcher and two or three doctoral/masters students.
- The criterion for funding is the novelty of approach and the likelihood of high-quality research being performed by the fundee, and his team.
- The likelihood of possible future application is NOT a criterion for making a choice between alternative/competing proposals.
- The primary expected end result of the research is a small group of qualified people whose expertise resulting from the research can be drawn upon to build an exploitable area of technology in the subject/topic area, and;
- The secondary expected end-result of the research is a body of knowledge which establishes (or rationally abandons) a potential application of a new, or inadequately understood, scientific principle.

'The knowledge base generated by the NRB through the above philosophy will be embodied in high-quality technical manpower and in the new techniques and design-tools developed through NRB-funding.'

Functions, powers and budget of NRB

- Support basic research that will generate new knowledge potentially useful to the Navy and to train young minds and hands to generate, use and apply that knowledge creatively to the purposes of the Navy.
- Set the principles and practices governing the relationship of DRDO with the academic world; 'think tanks', and non-profit research institutions in India and abroad as well as research strengths in the laboratories of the other agencies like CSIR.
- Consider, and approve, by peer-evaluation, research proposals submitted to the NRB. This evaluation will be accomplished through specialist panels.
- Set up research panels; set funding patterns and funding methodologies for the research panels of the NRB and approve their respective spending proposals for the year.

- Approve research programmes that overlap, or are not covered by, any research panel.
- Decide on specific research programmes or individual projects whose estimate of expenditure spread over the duration of the programme/project exceeds Rs. 20 lakhs, or involves expenditure on major civil works.

The guidelines define 'major' civil works as works 'which create permanent roofed area on land, or any structures in the sea'.

Collaterally, the NRB will fund the development of any specially-designed sensors, instrumentation, data acquisition and display systems necessary for carrying out the research funded by NRB. The techniques and tools of advanced simulation, and mathematical modelling will be brought to bear upon, and tailored to suit, the research programmes funded by the Board.

- ♦ NRB will also evolve methods to improve cooperation between Indian marine scientists so as to widen and deepen the benefits accruing from national projects through the exchange of expertise and knowledge and better use of facilities.
- ♦ However, NRB will exclude funding of the development of major systems like research sea craft and research submersibles (but will include funding of their use for the purposes of research funded by the NRB).

Of particular note in the 'Concept paper' is the new, innovative NRB programme on training and mobility of researchers, which reads:

'This programme aims to promote, through the training and mobility of researchers, an increase in the quality of human resources available to DRDO and to NRB-funded research programmes as also the enhanced use for these programmes of national ocean-related S&T facilities. The purpose of this programme of the NRB is to stimulate imagination and creativity by encouraging scientists to generate such research proposals to NRB as act as vehicles to serve the objectives below:

- ♦ to allow researchers to travel throughout India encouraging mobility between research-disciplines, between academic institutions, research institutes and R&D divisions of ocean-related industry;
- ♦ to help researchers to use existing large-scale ocean-related research, development, test and evaluation facilities and survey vessels;
- ♦ to improve the scientific and technological cohesion of the Ocean Research Community and increase the general level of scientific excellence in that community;
- ♦ to promote, through networks, the training of young researchers and trans-agency cooperation of scientists on research projects funded by the NRB.

It should be noted that the above are *objectives* of an NRB programme – not merely means.'

NRB programme on the dissemination and exploitation of research results

This NRB programme has as its objective the dissemination and exploitation of the results from scientific research funded by NRB so as to:

- ♦ ensure wide dissemination of research results;
- ♦ catalyse the exploitable areas of research results into innovations.

P. V. Indiresan, former Director, IIT, Chennai and currently Research Professor, Centre for Policy Research, Delhi, has been appointed as the first Chairperson of the NRB.

To fulfil its Charter, the NRB has been clothed with extensive executive and financial powers. Thus, the NRB has been empowered to rapidly identify and deploy on projects/programmes of the NRB 'high-calibre scientific and technical persons, predominantly young, identified both from within the country and abroad, provided they are not persons superannuated from the service of the Government of India or from enterprises. To this end, the NRB is empowered to offer fellowships and adjunct positions flexibly structured so as to maximize the interactions between academia, scientific laboratories or establishments, particularly those of DRDO, as also enterprises in the public and private sectors. These fellowships and adjunct positions may carry emoluments (personal to the individual holding the fellowship or position) not exceeding the maximum salary, and allowances & perquisites, of a head of a scientific agency of the Govt of India.'

Organize and support 'training and hands-on participation, for specified periods, in R&D activities in relevant areas in universities, R&D institutions in India and abroad and companies devoted to R&D'.

Support and fund, 'facility design and construction, including structures in the sea and the hiring of premises and seacraft within the limits of the relevant budget provisions'.

The budget for the NRB is expected to rise from the Rs 1.5 crores available for the second half of the financial year 1996–97, up to about Rs 5 crores per year in a few years.

DRDO sources point out, however, that: 'Money *per se* is unlikely to be a constraint. What is most worrying is the flight of young talent away from research careers in science and engineering. The NRB guidelines permit, in principle, even a 28 year old to receive a handsome fellowship of Rs 15,000 a month (plus accommodation) which figure will go up if and after the Pay Commission proposals are accepted by Government. The moot question is whether we can retain such a person in our national S&T system, even if not in DRDO, after his NRB fellowship terminates.'

Immune recognition

The Nobel prize for the year 1996 in physiology or medicine has been awarded to two immunologists – Peter Doherty and Rolf Zinkernagel for describing the manner in which the virus-infected cells are recognized by the immune system. Their work was published in 1974 from the John Curtin School of Medical Research, Canberra, Australia. Rolf Zinkernagel is now the head of the Institute of Immunology at the University of Zurich, Switzerland while Peter Doherty is an adjunct professor at the University of Tennessee College of Medicine, USA.

Defence reactions against foreign matter and the maintenance of individuality is a trait that has existed from the beginning of evolution and can be observed as incompatibility reactions in hydractinia corals as well as skin graft rejections in annelid worms. Elucidating the defensive role of cell-mediated immune reactions against pathogens began with the discovery by Elie Metchnikoff that macrophages were responsible for protection against foreign bodies in bipinnaria larva of starfish. Ever since, the idea of leukocyte involvement in immunity, has only been confirmed time and time again.

The conceptual need to evoke a distinction between self and non-self became apparent with the proposition of the clonal selection hypothesis by Macfarlane Burnet and David Talmage in 1957. According to this hypothesis, an individual is a mosaic of lymphocyte clones each of which is capable of producing only one type of antibody that is specific to a single antigenic determinant or epitope. Since an individual does not produce immunity against his or her own antigens, clones capable of producing antibodies against antigens normally present within the same individual need to be eliminated in the foetus – a time at which clonal diversification occurs. This is the process of tolerance induction to self molecules and therefore the need to distinguish self from non-self or altered self.

Using inbred strains of mice that they had generated by the 1920s, Ernest Tyzzer and Clarence Little established the presence of genes that controlled tumour rejection and tissue compatibility in general. During his efforts to describe blood groups in these inbred strains, Peter Gorer also observed that a group of genes controlled graft rejection. This gene complex was later termed the Major Histocompatibility Complex (MHC) when a similar set of genes were demonstrated in humans by Jean Dausset. It is now clear that the MHC codes for a set of highly polymorphic antigens known as MHC antigens and that these antigens are unique to each individual member within a species. Expression of these antigens on the tissue cells of one individual causes their recognition by lymphocytes when transplanted to another individual. Such transplants are also known as allografts. Lymphocytes belonging to different individuals recognize the presence of the other's MHC antigen (non-self) and react to it by enhancing their proliferation. It was this ability of lymphocytes to undergo proliferation and blast transformation that was used by Barbara Bain's group as well as Fritz Bach and Kurt Hirschhorn as the principle to develop the Mixed Lymphocyte Reaction (MLR) in 1964. Lymphocytes belonging to one individual termed as the responder are cultured along with irradiated lymphocytes of another termed stimulator cells. Irradiation of the stimulators blocks their proliferation but does not kill them. Hence the proliferating responder population can be followed by measuring the incorporation of tritiated thymidine into its DNA. Such assays along with other antibody-based methods are now used for tissue typing purposes.

The occurrence of cell-mediated cytolysis during graft rejections was also discovered by A. Govaerts at about the same time in 1960 while conducting kidney transplantation experiments in dogs. Lymphocytes from the recipient dog that had already rejected a donor kidney possessed the ability to kill the epithelial cells derived from the donor kidney. This basic observation later led to the perfection of the Cell Mediated Lympholysis (CML) assay.

The CML assay is basically a MLR that is carried out for 5 days. During this culture period, the unirradiated responding lymphocyte population is sensitized to the MHC antigens borne by the stimulator cells. Instead of adding thymidine, the surviving effector (killer) cells are added to lymphocytes or tumour cells matched at the MHC locus with the stimulators. These 'target' cells are labelled with radioactive chromium which will be released when the targets are killed by the activated cells generated during the culture. The use of radioactive chromium enables the quantification of the killing reaction. Thus the assay is based on the recognition of differing MHC antigens on the stimulating population by the responder lymphocytes. However, it soon became clear that CML can occur against many other membrane-bound antigens also.

Rolf Zinkernagel and Peter Doherty showed in their classic paper published in 1974 that Lymphocytic Choriomeningitis Virus (LCMV) would not be recognized by virus-specific cytotoxic T lymphocytes (CTL) if the LCMV-infected target was not matched at the MHC with the CTL effector cell[1,2]. Other target cells expressing MHC molecules different from that of the LCMV-infected stimulator cell were not recognized in spite of being infected with the same virus. In other words, CTL were blind to LCMV if they were not associated with self MHC molecules. This was termed as MHC restriction and was shown by Gene Shearer, Michael Bevan and others to operate for other antigens such as minor histocompatibility antigens, tumour-specific antigens and virus-specific antigens.

We now know that MHC restriction is due to the binding of antigen-derived peptides to the antigen binding cleft formed between the two α helices of the MHC Class I[3,4] or Class II molecule. The recognition of peptide-bound MHC on the surface of an antigen-presenting cell (APC) by the T cell receptor (TCR) makes MHC restriction a universal feature for most T cell interactions. The two subunits of the TCR contain immunoglobulin-like variable and constant domains that fold into a structure resembling the antigen-binding region of antibodies. Recent crystallization studies of the TCR-bound MHC Class I and viral peptide have now confirmed the

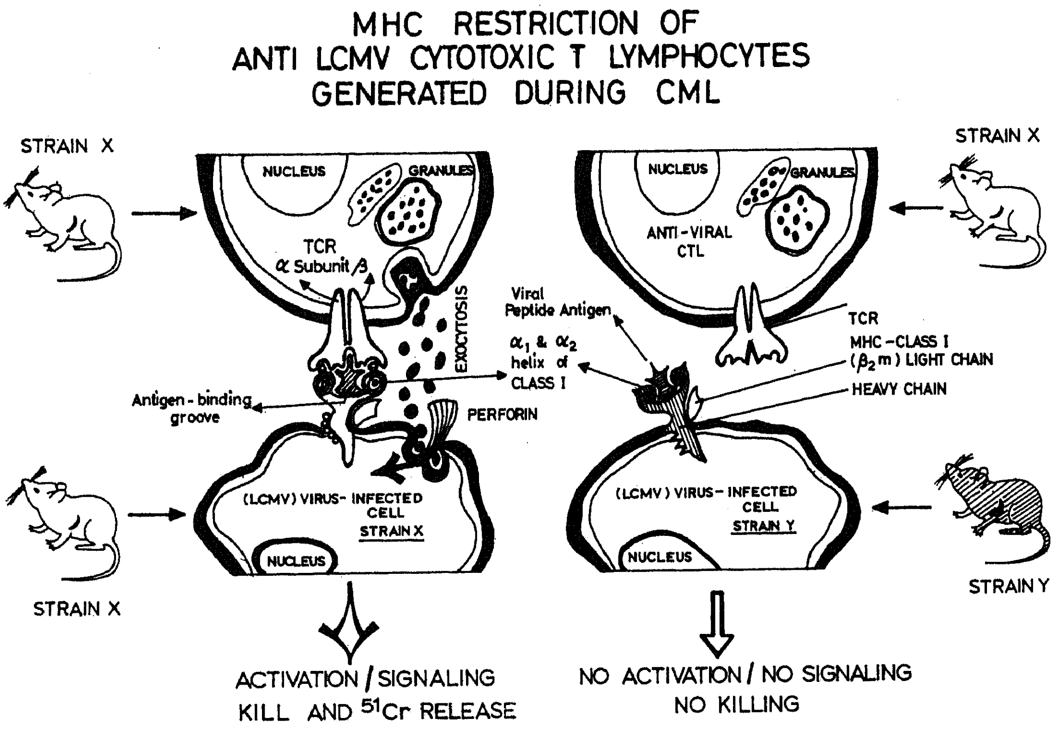

Figure 1.

phenomenon of MHC restriction in three dimensional terms. These papers[5-7] lucidly describe the quaternary structure of the TCR and show that a subunit of the TCR positions itself over the left end of the Class I antigen binding groove while the β subunit covers its right end. The α and β subunits thus make contact with residues in the amino-terminal half and the carboxy-terminal half of the antigenic peptide respectively. Confirmation that antigenic peptide occupies the antigen-binding groove of MHC has come not only from these studies but also from earlier interpretations of the electron density maps that were obtained therefrom[3,4]. The diagonal orientation of the TCR across the antigen-binding groove ensures that the first and second complementarity determining region (CDR) loops of a TCR subunit contact the amino-terminal part of the α_1 helix and the carboxy-terminal part of the α_2 helix of MHC Class I respectively. The TCR β subunit does the same in a complementary fashion, thus enabling the TCR to encompass both MHC and bound peptide in its face.

The affinity of peptide binding to the MHC molecule will be determined by the structural features of the peptide and the MHC allele that it binds to. Different MHC antigens bind different peptides. Thus T cell activation is not only influenced by MHC and peptide but also by the manner in which this complex is recognized by the T cell receptor. It could be speculated that the generation of antigenic peptides by APC could also differ between individuals depending on the manner in which different proteins such as TAP and LMP proteins function during antigen presentation. Therefore the development of most vaccines and therapeutic procedures against infectious as well as autoimmune diseases will be influenced by the discovery of MHC restriction. It is now easy to understand why the award of the Nobel prize to Rolf Zinkernagel and Peter Doherty was well deserved.

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Research snippets (*compiled by A. V. Sankaran*)

Neutrino and muon bursts wiped out dinosaurs

During the long history of evolution of life on Earth, there have been periodic mass extinctions of some of the life forms. Many causes, terrestrial and extra-terrestrial have been advanced for their exit, particularly following the publication of the well-known meteorite impact theory[1] in the eighties. The latest agent of extinction invoked is – cancer-causing mutations triggered by massive bursts of neutrinos released by the dying stars in our galaxy[2], or by energetic muons from neutron stars[3].

Theoretical calculations about the damage that can be caused to living tissues when neutrinos collide with the cell nuclei have shown that they can be quite harmful. Neutrinos normally do not interact with matter but under sudden flood of their emission during stellar collapses, such collisions can take place, and in fact this has been happening within 20 light years of Earth almost every 100 million years. The collision results in recoil of nuclei which damages the DNA, inducing cancer-causing mutations; according to calculations, each recoil may deposit more than a thousand electron volts of energy along a track length of 10 nm, which is a substantial amount – about 12 malignant cells per kilogram of tissue – a damage quite severe considering the fact that dinosaurs are large bodied.

Recently, three astrophysicists from Israel, Arnon Dar, Ari Laor and Nir Shaiv of the Technion in Haifa, have also expressed similar views[3] and feel that mass extinctions during past geological history could have been triggered by the collision of neutron stars. Such collisions could deliver in a single day equivalent of 100,000 years' worth of rays which on hitting upper atmosphere of earth could generate a cascade of highly energetic muons capable of killing organisms directly, apart from destroying ozone layer and making the environment radioactive. However, since muons are stopped by rock and a few tens of meters of water, organisms living below these levels are shielded and survive.

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Vagrant North America and its drop out

Ever since Eduard Suess, the Austrian geologist first postulated the giant Gondwanaland, a primeval amalgamation of all the early landmasses, a concept which soon led to the development of the well-known hypothesis of Continental Drifts by Alfred Wegner, geologists have been re-examining and revising the concepts of global palaeogeography. By early sixties, the Wegnerian ideas of continental drifts, which had remained dormant for some decades, were revived with the advent of global plate tectonic theories developed by notable geologists of those times like Hess, Vine and Mathews. According to these theories, continents grew by accretion of crustal fragments and mid-ocean islands; the continents could also break, and segments of such crusts could migrate across oceans. Based on data from stratigraphy, internal structures, fossils, palaeomagnetism, seafloor explorations, mantle dynamics, computer simulations supported by satellite and seismic records, the peregrinations of the continents during the geologic past became clearer, though some of the new thinking about the palaeogeography of the ancient continents are still much debated.

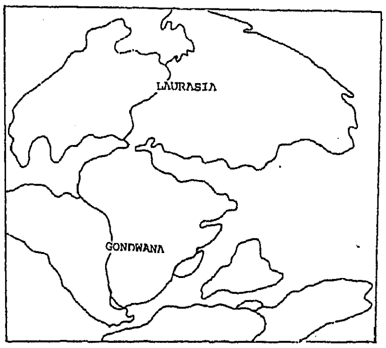

Figure 1. Pangea, 180 million years ago, showing break up into a Northern Laurasia and Southern Gondwana.

Views about the ancient continental dispositions, as a single vast land called 'Pangea', during the Permian times (250 million years ago) and how it split into 'Laurentia' (North America, parts of Europe and a few other continental fragments) and 'Gondwanaland' (Africa, South America, Antartica, Australia, India) during later times are today well known[1] (Figure 1). But the palaeogeography prior to this period, during Palaeozoic and Precambrian times, is difficult to conceive in the absence of ancient ocean floors to provide guidelines. During the last ten years, some attempts at this exercise have yielded a fair picture particularly about the wanderings of the North American continent. This has been deduced from a study of palaeomagnetism in the iron-bearing minerals formed at different periods on this continent. These minerals orient themselves along the magnetic N–S direction (or more precisely, the geomagnetic field) when they crystallize from the magma, and a critical evaluation of their orientation in the rocks formed at different time intervals provides reliable data to infer about the latitudes the continent occupied (wanderings) in the past.

According to a hypothesis[2] presented a few years back called the SWEAT hypothesis (for SW U.S – East Antartica) based on field trips to Antartica by Eldridge M. Moores of the University of California (Davis) and Ian W.D. Dalziel at the University of Texas (Austin), the Shakleton Range of Trans Antartic Mountains expose rocks of similar characters and age (1.8 billion years) as those lying under New Mexico and Arizona or those of Grenville Province on the eastern and southern margins of North America. Hence they believe that these areas must have been juxtaposed at one time during the past eons. This juxtaposition of Laurentia (ancient North America) and East Antartica prior to

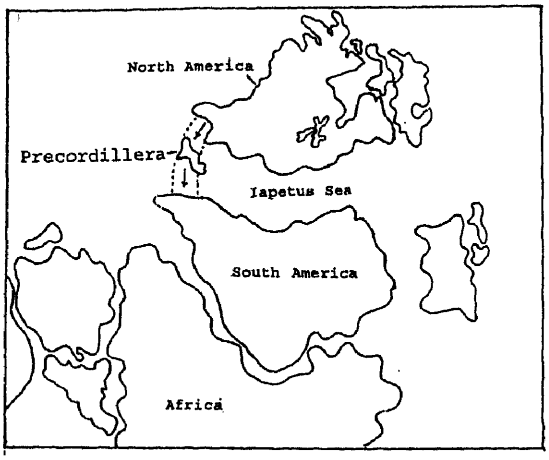

Figure 2. Breakaway microcontinent (Precordillera) adrift in the Iaepetus Sea to dock later with South America (alongside present day Andes).

the formation of Pangea, according to them, could indicate existence of an earlier megacontinent which they called 'Rodinia', a fusion of Antartica, Australia, North America and fragments of South America. The oceans that existed at that time between other continents – India and Africa and Africa and South America were destroyed between 750 and 550 million years and all nucleii of Africa, Australia, Antartica, South America and India amalgamated into the giant 'Gondwanaland'.

They further argue that Laurentia, the ancient North American continent, as per magnetic data, wandered during the late Precambrian and Palaeozoic times around the west coast of South America and collided with it at a place where Andes was pushed up much later and finally, when Pangea was formed, abutted against northwest Africa pushing up the Appalachian mountains in the process. This Laurentia–South America collision during Palaeozoic was also strongly supported by Louis H. Dalla Salda from the Argentinian University of La Plata, on the basis of tectonic and faunal studies in the Andes. Though these inferences about the journeys by the ancient North America were based on computer simulations by Dalziel, he hoped that future discoveries of land fragments, ripped-off continents and accreted to other landmasses, will soon come up in support of his views; and, indeed, they came up soon.

The first such accreted fragment was discovered in the foothills of Andes, as a tract of limestone 100 miles wide and a 1000 miles long, called Precordillera, north of Mendoza in western Argentina. This tract appeared a misfit here and had distinctly different lithology and fossil content with respect to the rest of the surrounding areas. According to Victor Ramos[3] of the University of Buenos Aires, Argentina, the occurrence of distinctly marine trilobites as well as pillow lavas (rocks which form when submarine lava eruptions congeal rapidly) on both sides of the Precordillera would suggest that this segment must have been once surrounded by sea before it became part of South America. He also felt that this piece of land might have broken off the ancient North American east coast (now the southern USA, parts of Texas and Arkansas) some 500 million years ago and drifted to its present position along the Andes mountain range.

The above view recently received strong support from William Thomas of the University of Kentucky and Ricardo Astini of the University of Cordoba, Argentina[4], who found that 'Cambrian fossil fauna of the Precordilerra were identical to those of continental shelf succession of Laurentia and Cambrian succession of Precordillera similar to those of margins of Laurentia'. They also felt that this piece of land must have rifted away from Laurentia (the Ouchita rift), during the Cambrian times and drifted as a micro-continent across the intervening sea (Iapetus Sea, now extinct) as an island before berthing alongside west coast of Gondwana (South America), during the Ordovician, some 80 million years later (Figure 2). Till the time this piece of land was close enough, faunal exchange with mainland Laurentia was taking place; but when the gap widened with time, this segment had its own unique younger fossils which were absent in Laurentia (North America) and later when close to Gondwana (South America), faunal exchanges resumed. They feel that these features strongly support the view that the castaway Laurentian (North American) terrane spent some time as an island before berthing with the west coast of South America; this implies that Laurentia and South America were not in direct contact, a view that questions the direct collision of the two as advanced by Moores, Dalziel and Louis Dalla Salda. There is also a third view[5] that a submarine plateau may have attached the Precordillera to North America. Geologists are now looking for similar castaways – rifted continental segments or terranes – that had accreted to other continents in the globe. Tentatively, they have detected two such possibilities in the rock tract in Piedmont Mountains in North Carolina which appear to be a part of South America as also Oxaco region of southern Mexico.

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2. Dalziel, I. W. D. *Sci. Am.*, 1995, **272**, 38–43.
3. Ramos, V. A., Jordan, T. E., Allmendinger, R. W., Mpodozis, C., Kay, S. M., Cortes, J. M., and Palma, M., *Tectonics*, 1986, **5**, 855–880.
4. Thomas, W. and Astini, R., *Science*, 1996, **273**, 752–757.
5. Kerr, R. A., *Science*, 1995, **270**, 1567–1568.

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The 'Vision 2005: Earth Sciences' document of the Department of Science and Technology was recently printed in the pages of this journal (Curr. Sci., 1996, 71, 820–823). An alternative view, espoused by one of India's leading geologists is reproduced below.

– Editors

Priorities in earth science research

B. P. Radhakrishna

'If we want the world to pay greater attention to geology, and if we want decision makers to allocate more resources for geology, then we need to demonstrate the importance of geology in public affairs and we must accept our public obligation to be good citizen geologists.'

E - an - Zen

As the twentieth century is drawing to a close, scientific institutions in the country have taken to the exercise of presenting 'vision papers' outlining the goals of research to be pursued by them. The United Front Government at the Centre too has come out with a Common Minimum Programme (CMP) to be followed during the coming years. It is worthwhile to examine the relevance of these programmes and the role of Earth Science in the present context.

One of the vision documents lists the following areas of opportunities for research in the field of Earth Science: (1) Evolution of the Indian Crust, (2) Himalayan Orogenic Belt, (3) Earthquake Problems, (4) Reconstruction of Palaeoenvironments and (5) Earth Science Application for Societal Needs.

Are these the priority areas of our research? What are we really going to achieve? Do these in any way contribute to the welfare of our people? If such questions are posed, it will be seen that a good part of our research effort is aimless and that we are trying to answer questions which nobody is asking.

The trouble with our research programmes appears to be that our top research personnel occupying senior positions in our universities and research institutions have received training in universities of UK, Europe and America. So trained they cannot but pursue the type of research that is being practised in those countries. Their vision has seldom extended to finding solutions to the problems faced by this country. Science and Technology have yet to become effective tools for national growth and economic development. The time appears ripe for introspection and rethinking.

The glaring fact remains that this country, even after fifty years of independence continues to be one of the poorest nations in the world. It is not as if we are lacking in resources. For its size, India has a variety of mineral and metal resources to satisfy many of its needs. Compared to other parts of the world it receives copious amounts of rainfall. Its soil is fertile. Hill slopes are covered with evergreen forests. The country is populated not by aborigines but by intelligent people with thousands of years of culture behind them. Its human resources are second to none. And yet, in spite of all these plus points, we remain poor. The reasons are not far to seek. It is because we seem to be wasting our time in imitative and aimless research and serious problems facing the country have been largely ignored. Have we tried to identify gaps in our knowledge and pose the right questions? First and foremost there is need for educating the public and those who are occupying seats of power as to what the scope of Earth Science is and what it can do to better our economic well-being.

Energy needs

As a consequence of the big boost given to the growth of the automobile industry, there has been an alarming increase in the number of vehicles and consequently in oil consumption which stands at 65 million tonnes per year. Indigenous production has remained stationary at around 35 million tonnes, necessitating import of the balance quantity of 30 million tonnes per year! This drain is likely to increase in the years to come, causing a big hole in foreign exchange resources amounting to over 7 billion dollars, or nearly 25,000 crore rupees.

Sedimentary basins having a good potential for oil cover an area of 1,400,000 km^2 on land and 720,000 km^2 offshore. This is an indication of the magnitude of the work before earth scientists – geologists and geophysicists alike – in carrying out detailed exploration of these sedimentary basins with a view to identifying favourable structures for test drilling. No concerted effort is yet evident in this direction. We seem to be waiting for others to come and do the job for us. The zeal and enthusiasm which were in evidence at the time of the formation of the Oil and Natural Gas Commission are lacking today. If goals are set and our scientists motivated and encouraged, there is no reason why India should lag behind in attaining self-sufficiency in respect of her oil requirements. One need not always be on the look out for giant fields, the many smaller fields also require attention.

The same lack of will is evident in the development of natural gas resources. Adequate attention is yet to be directed towards wise utilization of the identified reserves. In any agenda for research in the coming years, the identification of fresh resources of hydrocarbons should, therefore, occupy the first place. Geophysicists and geologists have a challenging task before them in these areas alone.

There is quite a bit of loose money in the country as is revealed by the insatiable thirst for possessing gold. Annually not less than 25,000 crores are being diverted in this way for want of better avenues for investment. Government policies and private initiative should aim at providing incentives to wean people away from investing their surplus money in gold and divert it for nation-building activities.

Reprinted from *J. Geol. Soc. India*, 1996, **48**, 493–496; 611–616.

Mineral resources

We do not yet have a comprehensive volume on the Mineral Deposits of India to enthuse our young men about the immense possibilities that exist in the development of our mineral resources. The Mineral Year Book brought out by the Indian Bureau of Mines falls very short of what is actually required. The geological setting of the country holds great promise for a boom, in mineral production and in order to usher in such a boom, regional mapping programmes have to be accelerated in order to stimulate mineral exploration. Proving extension of known ore bodies, identifying new ore zones and discovering new satellite ore bodies are items of research which should be encouraged and adequately funded.

Gold production has remained at a miserably low level. There is no urge or incentive to produce more. Hundreds of promising occurrences which should be humming with activity are left undeveloped, denying large-scale employment opportunities to the rural poor.

Utilization of resources

The practice of exporting large quantities of iron ore, manganese ore, chromite, barite, etc., without any processing to add to their value is still continuing unabated. This will not bring any prosperity to the country and requires to be stopped. The setting up of iron and steel production units is a step in the right direction and these industries have to be helped in obtaining an assured supply of suitable raw materials. This in itself is a major task which could provide unlimited employment opportunities to earth scientists.

Decentralization of powers

The CMP of the Government of India does not make any reference to the development of mineral resources. It, however, recommends devolution of powers to the States. This is a welcome move, as at present all power is centered in Delhi. Decision making is avoided at all levels, making the task of obtaining a mineral concession a most frustrating and exasperating exercise. The spate of applications received as a result of the liberalization policy announced by Government more than two years ago are still lying in cold storage with absolutely no action taken on any of them. There is no reason why State Governments, which are keen on developing the resources of their respective States should not be empowered to decide on the grant of mineral concessions subject to strict observance of rules and regulations in this regard.

Close association with industry essential

Official Survey Organizations, Universities and Research Institutions should forge greater links with industry and not function in isolation. Considerable amounts are being spent by several government organizations on exploration without first identifying the exploiting agency and follow-up action on mining and mineral processing is conspicuous by its absence. There is great apathy both in government and the private industrial sector, towards mining and exploitation of mineral resources. Need-based research should receive greater emphasis and be financially supported. Projects undertaken by research institutes and universities should be oriented towards providing the industry with new and more efficient technologies.

Subsequent exploration and exploitation should be in the hands of a large number of private companies and individuals and not in the hands of governments as at present. Official survey organizations can play an important role providing the industry with detailed geological information, maps, and conceptual models, which will prove helpful in these areas and help the quest for new exploration concepts and in the discovery of new ore bodies. Key centres of research in economic geology should be developed where Government, Universities and Industry shall work in close co-operation. Increasing amounts should be made available for research in this field.

Every project undertaken by the official survey organizations should end with a detailed report, which should be readily available to interested exploratory organizations and persons and not kept in cold storage as at present. Official survey organizations should also provide effective, responsive and efficient customer service. There is no harm in throwing open the country, with adequate safeguards, to international exploration, as all efforts at resource identification and exploitation should be encouraged. It is a never-ending process which, fuelled by its own successes, will gain momentum.

This does not mean that basic research should not be encouraged, but that is best confined to a few specialist groups. Repetitive and imitative research on the same type of problem by different Institutes and University Departments will not produce results commensurate with the time and money spent.

Significant progress possible

That India can make significant contributions in the field of science and technology is borne out by the spectacular results achieved by agricultural scientists in bringing about the green revolution and making the country self-sufficient in its requirements of food grains. Earth scientists too, can make an equally striking contribution if only they go about it in the right way, identifying resources and developing the skills for utilizing them to satisfy the Nation's requirements.

If spectacular progress can be achieved in highly specialized fields like software technology, cold fusion, and adventures in space, there is no reason why India should lag behind in developing her mineral industries and produce real wealth. What is needed is massive co-ordinated research programmes with the provision of adequate financial support unhampered by bureaucratic control. It is not the lack of instruments that is retarding progress but the lack of a will to succeed. Making a start is half the battle won.

Water-related problems

The ugly spectacle of feuds that are raging between States over sharing river waters is before us as a standing warning as to what is in store for us in the near future. Most of the problems that the country is presently facing appear to be the result of mismanagement rather than lack of resources. There is enough water to satisfy our needs but, who can place a limit to human greed?

Our knowledge about the water resources of the country and the extent of their utilization is far from satisfactory: Take the water needs of a city like Bangalore – we have no information on what percentage of population of the city depends on municipal water supply, how many depend on groundwater for their needs and how many live by purchasing water from dealers? State Groundwater Boards do not have correct records of the number of bore-wells that exist and the quantity of water pumped each day. No limit is placed on the exploitation of this essential and precious resource. Every day we hear of large sums being sanctioned for sinking new bore-wells without first considering the effect of large-scale withdrawal on a shrinking resource. An alarming decline in the water table has not in any way dampened the zeal for new wells, and hardly any attention is given to the replenishment of the resource through recharge. Forests which had the effect of arresting rainwater run-off and recharging groundwater reservoirs are being destroyed, leading to floods and severe soil erosion. River channels and surface water reservoirs are getting choked with silt. No effort is made to impound rain water and allow it to percolate down and recharge the groundwater reservoir. The extent of work awaiting the attention of geohydrologists is enormous.

Nature has provided a vast interconnected reservoir of water below ground. Ways of replenishing this reservoir should be our main concern. Attention has to be given to terracing, contour bunding, creation of farm ponds and afforestation of catchment areas. Such practices have the aim of arresting the free flow of rainwater and allowing it to infiltrate below ground and enrich the groundwater reservoir. Scientific knowledge has to be put into practice in demonstration plots and the message of water conservation conveyed to the public. The scope for service in this field is unlimited.

The advent of down-the-hole hammer rigs capable of drilling rapidly, even through hard rock, and the deep-well turbine pumps capable of lifting enormous quantities of water have revolutionized agriculture. They have, at the same time, allowed unlimited withdrawal of groundwater far in excess of annual recharge. Technology, while helping the farmer, has tended to jeopardize and possibly ruin a precious resource through overuse.

Withdrawal of groundwater in excess of the annual recharge should not be permitted under any circumstances. It will lead to a lowering of the water table and result in every other well in the region going dry. This is already happening in many parts of the country and if this practice of unrestricted utilization continues, there will be a water crisis in the not-too-distant future. When that happens, water will not be available even for drinking, let alone for irrigation.

The extent to which surface water and groundwater are increasingly polluted as a result of the use of pesticides and fertilizers is another major problem. Remedial measures to prevent chemical pollution of the precious resource must be taken without delay.

Educating the intelligent farmer, making him understand the problems he has to face and acquaint him with appropriate remedial measures he has to take to protect this resource is the paramount need of the hour.

It is earth scientists who are primarily concerned with the study of all aspects of groundwater – its occurrence, quality, exploitation and conservation. Unfortunately Research Institutes, and University Geology Departments have given a low priority to the study of groundwater-related problems. This is the one field where they can come into close contact with the people and in which, they can, by the application of their scientific knowledge, contribute to public welfare. Earth scientists can have all the job satisfaction they need, when they realize that they are of some service to their fellow men.

Surface water resources

Surface water resources too are not being used wisely. Vast sums of money have been spent in building high dams and storing large quantities of water. In this process, very large expanses of valuable agricultural land have got submerged, causing wholesale displacement of local populations and consequent untold misery. While engineering skill of a high order is evident in the construction of major reservoirs, the same technological skill has not been displayed when it comes to the proper utilization of the stored water. Methods presently adopted for conveying water are the most primitive and wasteful, in no way different from the practices followed thousands of years ago. Irrigation, as a result of such wasteful practices, remains the largest sick industry in India today with hardly any return on the huge capital invested. Major irrigation projects stand as examples of wasteful utilization of a precious resource, with no regulatory structures and measuring devices for supply of water. No value is fixed for the water supplied, resulting in wasteful utilization, far in excess of what is actually required. In this process, fertile land is also getting degraded, waterlogged and saline.

In matters of water conservation much can be learnt from Israel. Water in that arid country is an exceedingly precious commodity calling for maximum conservation. It is conveyed through pipes to prevent loss through seepage and evaporation and meters are installed to regulate supply with no wastage permitted. Sprinkler and drip irrigation are practised on a large scale in order to obtain maximum benefit from the water. We, on the other hand, by continuing obsolete and wasteful practices, are conveying water in open cut trenches using only 30% of the stored water in crop production. In the face of a rapidly growing population and the need for growing more food, we can ill afford such amateurish and unscientific utilization of water.

Water is the most precious of our natural resources and has to be conserved and used with great care. Effecting improvements in our irrigation practices is a realizable goal which has to be implemented without delay. Yields under flood irrigation, as practised in India, are too low, less than one tonne per hectare, while Japan, with controlled irrigation, has been able to produce 5.5 tonnes and in some cases as much as 8 tonnes per hectare. This is an indication of what is possible.

There is, again, a craze for planting water intensive crops like sugarcane. It should be remembered that water required for one hectare of sugarcane crop can irrigate 10 hectares of wheat. Scientific planning is necessary in making use of limited water resources and drawing maximum benefit.

Surface storage even in our largest dams is far from dependable. Dams can at best store one year's supply only and cannot safeguard against years of drought. Greater attention has to be given to groundwater reservoirs as their storage capacity is considerable and they can help tide over years of poor rainfall.

Those who benefit from major irrigation works are the richer and more articulate sections of society. The water need of the large majority of the population, living in regions far away from perennial sources and affected by droughts almost every year, is sadly neglected. No single Research Institute, to my knowledge, has specialized in water resource management and the country may have to pay a heavy penalty for neglecting this most important field of research.

Land use planning

It is estimated that there are 142 million hectares of arable land, of which only 46 million hectares have been brought under agriculture, the rest lying barren. The country cannot afford to allow such vast extents of land to lie barren. Science and technology aided by the intelligent use of modern mechanical devices should bring much of this land lying fallow under the plough. Terracing of land, excavation of contour ditches, erection of bunds, afforestation and all such land-use practices aimed at arresting free surface flow of rain water and making it percolate into the ground are needed.

Private industry and business establishments should take to farming and organize agriculture as an industry. Large investments are needed for developing infrastructure like roads, wells, drip irrigation pipe lines, pump houses, fencing and drainage. Modern technology has to be adopted with a view to seeing that every drop of water is utilized and the precious resources never allowed to run waste. Such development can bring about a major revolution in irrigation through planned groundwater exploitation.

Research on a variety of topics related to use of surface and groundwater can be suggested. Soil geochemistry, soil productivity, estimation of optimum requirement of water for different crops, estimation of evapo-transpiration losses, soil fertility, effect of irrigation on groundwater regime, conjunctive use of surface and groundwater, efficient water management, irrigation water-use efficiency, formulation of irrigation methods aimed at water conservation, salinity, prevention of soil degradation through excess irrigation – all these need expert attention. The problems are complex and require the combined efforts of hydrogeologists, irrigation engineers, and soil scientists. What is more important is that the results of research should be continuously communicated to the farmers, making it easy for them to grasp the details and enabling them to adopt new technology for better water management and improving agricultural efficiency.

Agricultural scientists have shown a better sense of responsibility. Instead of wasting their time in inappropriate research, they have adopted down-to-earth policies and deviced innovative measures to improve the conditions of the farmer. There has been a phenomenal increase in food production and very soon the country may become an important exporter of food grains. The most advanced research is being directed to produce hybrid seeds and step up food production. This is because, the large majority of agricultural scientists are engaged not in aimless academic research but on practical research oriented towards finding solutions to the problems faced by the farmer.

Environmental problems

There are several environmental problems which only geoscientists can solve, for they have the necessary geoscientific knowledge gathered over a period of years. Areas of special interest are: waste disposal, threat of natural hazards like floods, landslides, earthquakes, desertification, coastal erosion, etc.

How to improve the quality of life of our people should be the primary concern of present and future geoscientists. Geology, it should be emphasized, is a social science and not a laboratory oriented discipline. The whole earth is its laboratory and human welfare its goal. Playing down field work and glorifying laboratory-oriented research has been the root cause for the decline in pubic appreciation of the value of geologist's services.

Environmental considerations should not, however, come in the way of progress by putting obstacles in the development of resources aimed at improving the quality of life of the common people. A balance has to be struck between stepping up mineral production and preservation of environmental quality.

Quaternary studies

Processes which have operated on the earth, especially during the last 10,000 years (Holocene), need special study. Information on rates of erosion, sediment transport and accumulation in standing bodies of water, is lacking. Sediment cores from silted-up reservoirs offer the best material for study as they preserve a faithful record of environmental changes.

The Himalayan terrain, which is environmentally fragile, requires special attention on the part of earth scientists. The evidence afforded by river terraces and the distribution of plant and animal life point to uplift of the terrain even after the advent of man. Rivers have changed their courses, transforming fertile lands into deserts and *vice versa*. Deformation as a result of uplift and movement along faults has made the area particularly prone to landslides. Floods are causing enormous havoc almost every year. Earth scientists, through detailed field studies, should be in a position to develop prognostic capability, identify areas prone to natural hazards and forewarn the people. This is another field of research in earth science which should be undertaken on a priority basis.

Study of soils and soil forming processes

India has the largest accumulation of fertile black soils, specially in the States of Maharashtra, Madhya Pradesh, Karnataka and Andhra Pradesh. The role of parent rock and changing climatic conditions in soil formation has not been clearly understood. A clear understanding of processes leading to soil formation, reasons for degradation of soil and scope for regeneration and increasing soil productivity, should form an important branch of earth science research.

More important than dating rocks formed thousands of million years ago is the dating of geologically young materials like soils. Information on the time required for soil formation is lacking at present. New techniques, using isotopes produced by cosmic radiation, being applied elsewhere to solving current problems can also be attempted here for better soil management and conservation.

Palaeontological studies

Palaeontology, the study of ancient forms of life, is at the very core of earth science studies. The main contribution of geology and its greatest triumph has been the decipherment of earth history during the past 600 million years. In spite of this enviable past record, palaeontology is today a neglected field of study.

The zonal classification of strata, using macro and micro fossils, ought to be a continuous process refining the stratigraphic record. Such studies of samples collected from off-shore wells and cores recovered from the ocean bottom and deep drilling projects have great practical importance in the exploration for hydrocarbons. Modern technological aids like electronic imaging system and electronic data bases have greatly enhanced the value of palaeontological studies. Palaeontology combined with seismic stratigraphy can be a powerful tool in providing chronological control. Major global environmental events can be recognized more precisely by the appearance and disappearance of organisms.

Geological Survey Organizations at State level and University Departments teaching earth science should take the initiative in organising natural history museums, with ancient life forms on display, kindling interest in the minds of children and the lay public. The success of *Jurassic Park* and the crowds of visitors attracted to view life-size, models of dinosaurs indicate the extent to which the public can be made to realize the importance of geology in understanding the history of the evolution of the earth and its inhabitants.

The foremost need of the hour is to do everything possible to increase productivity in our agricultural land and in our industrial establishments. The all-pervading bureaucracy should reorient its thinking and develop a helpful, instead of an obstructionist, attitude. The inferiority complex that is apparent in many of our young men should be replaced by confidence in our ability to develop our resources to our advantage.

My main object in drafting these notes is to focus attention on the need for geoscientists to bestir themselves and strive their utmost to increase their knowledge about land, mineral and water resources and be of service to the people. Societal obligations should gain precedence over purely professional recognition. Subsidies are not going to improve the living conditions of the rural poor but will kill all initiative and condemn them to live on perpetual doles. What is needed is employment opportunities and assurances of adequate income. This is possible only through development of land, mineral and water resources on a scientific basis.

The President of the Geological Society of America in his recent Presidential Address posed the question 'If geoscientists went on strike, would anybody notice?' Our geoscientists should likewise ask themselves – 'how relevant is my research to the needs of the country and the betterment of the people?' In the final analysis 'the support of the geologist depends on public appreciation of the value of his services'.

'Do you wish to be a patriot? – then tune yourself in love with your country and the people.' – Swamy Rama Tirtha.

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Academy initiative in university education

The Indian Academy of Sciences, as a part of its initiative in university education, has limited funds available to support:

- Short visits (4–8 weeks) by college/university students and teachers to Academy Fellows for joint work on specific projects.
- Short intensive lecture series by Fellows in colleges and universities.

Proposals are invited by the Education Panel of the Academy (R Narasimha, Chairman) for the above from college and university teachers and students. They should include a brief resume, a one page description of the proposed activity including its duration and tentative dates, and a letter of consent from the concerned Fellow, and should be mailed to: *Executive Secretary, Indian Academy of Sciences, PO Box 8005, C V Raman Avenue, Bangalore 560 080.*

CdS nanocrystallites: An efficient light emitter

When wide band gap semiconductors with appropriate doping are exposed to light with energy larger than the band gap, it may so happen that it emits visible light. In order to qualify as an efficient light-emitting material, a semiconductor must have band gap ~2.0 eV. For many years, wide band gap II–VI compounds, viz. ZnS, ZnSe and CdS have been good candidates for such light-emitting devices under UV and electron beam excitation. In principle, the band gap can be varied by changing the crystalline size. As the crystalline size decreases, the band gap value increases. Thus, for low band gap material, one has to control the crystalline size to obtain the required band gap value.

Efficient light emission has already been reported for Mn^{2+} doped ZnS (ref. 1) nanocrystallites synthesized by polymer caping technique. Essentially, favourable energy levels within the band gap of nanocrystalline ZnS are created by doping for efficient luminescence transfer. It emits yellow light similar to the luminescence from bulk doped ZnS. What changes is the efficiency of the process. The efficiency changes from 0.3% in bulk to 18% in nanocrystallites, leading to a tremendous improvement in the brightness of emitted light. Because of the small size, the intensity of emitted light becomes more.

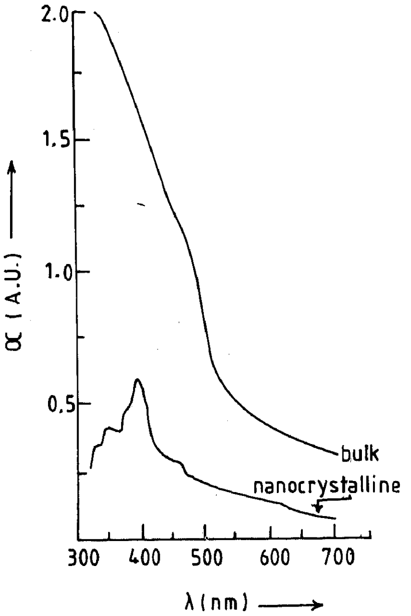

Figure 1. Room temperature optical absorption spectra of CdS semiconductor of different crystalline size.

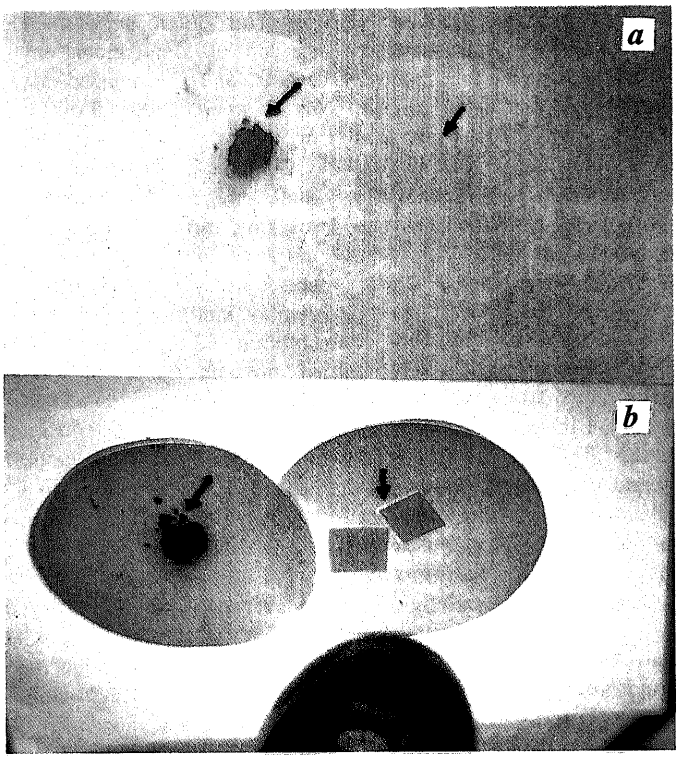

Figure 2. Optical colour photographs of visible light emission from CdS on filter paper taken at 300 K. *a*, Photograph taken under visible light. Orange colour crystallites are bulk CdS with band gap 2.405 eV and average size 75 nm. Faint yellow crystallites have band gap 2.97 eV and average size 5.0 nm. *b*, Photograph taken under UV radiation for samples as in *a*. The nanocrystallites emit visible red light whereas the bulk CdS does not.

We have prepared a batch of CdS samples of different crystalline size by a chemical bath deposition technique[2]. Optical absorption spectra at 300 K of two CdS samples (bulk and nanocrystalline) are shown in Figure 1. The band gap for these two samples is found to be 2.405 eV and 2.97 respectively. It is noted that in case of nanocrystalline CdS film there are several absorption maxima in the form of oscillations which is a signature of the size quantization effect. From the blue shift[3], the average radius of CdS nanocrystallites was estimated to be 5.0 nm. Depending upon the crystalline size, we could change the band gap from 2.4 eV to 2.97 eV which is significant.

Since our nanocrystalline CdS semiconductor has band gap 2.97 eV, it has to be doped to achieve light emission. However, Rutherford Back Scattering analysis show excess Cd in the nanocrystallites, which in principle should act as autodopant in CdS (ref. 2). Thus, if these nanocrystallites are now excited with higher energy photons, one expects to see visible light emission whose colour would depend upon the crystalline size and the trap levels present within the band gap of the nanocrystalline semiconductors. The optical colour photograph of bulk CdS powder and nanocrystalline CdS prepared on filter paper taken under visible light is shown in Figure 2 *a*. The orange colour powder corresponds to bulk CdS of crystalline size ~75 nm and the faint yellow colour slices corresponds to nanocrystallites of size ~5.0 nm. The same samples are exposed to UV light at 300 K and the photographs are shown in Figure 2 *b*. It is observed that the bulk-CdS powder does not emit any light and look black whereas the nanocrystalline samples emit red light. Our CdS nanocrystallites are unique of their kind – which clearly demonstrates the possibility of light emission only from such nanocrystallites of average size 5.0 nm. Thus, our CdS nanocrystalline materials can now be used as an UV sensor.

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Xylanase production and its impact on bleaching of hardwood kraft pulp

Traditionally, wood pulp used for paper making process is bleached using chlorine or its derivatives such as calcium hypochlorite and sodium hypochlorite to remove residual lignin present in the pulp. The main cause for brown colour of the pulp is the presence of residual lignin. Chlorinated organic compounds produced during conventional pulp bleaching are detrimental to environment and highly resistant to microbial degradation. Chlorinated organic compounds are mainly responsible for the discharge of AOX (adsorbable organic halogens) in the receiving waters[1]. Modern techniques such as prolonged kraft cooking, bleaching with hydrogen peroxide, oxygen, enzyme (xylanase) and ozone have been introduced recently to reduce the production of chlorinated organics during bleaching[2]. Among the new techniques, enzyme bleaching is becoming important in the recent past due to its environmentally friendly nature[3–5]. In kraft pulping, xylan, one of the major components of wood hemicellulose, is solubilized during the initial stages of the cooking period and in the final stages of pulping, xylan gets precipitated and redeposited on the secondary cell wall of the fibre. The redeposited xylan acts as a bonding material between residual lignin and cellulose present in the pulp[4]. Therefore, limited hydrolysis of the xylan present in the pulp by hemicellulase enzyme like xylanase was found to improve the bleaching performance, reduce AOX discharge and active chlorine consumption in bleaching process[3,6–10]. Therefore, efforts were made to produce xylanase enzyme and apply to mill-made hard wood kraft pulp in our laboratory.

Table 1. Optimized conditions for the production of xylanase enzyme by *Trichoderma* sp.

Parameter	Condition
Corn cobs	15.0 g/l
Peptone	15.0 g/l
$(NH_4)_2SO_4$	2.0 g/l
KH_2PO_4	5.0 g/l
$MgSO_4$	0.3 g/l
$FeSO_4$ ($7\,H_2O$)	0.25 g/l
pH	6.5
Temperature	Ambient
Tap water	1000 ml

Several fungi were isolated from the stored bagasse from the bagasse yard at TNPL mill site using malt extract agar medium. All the fungi were screened for xylanase production using culture medium containing 1% xylan from bagasse chemical pulp[11], 0.5% peptone, 0.2% $(NH_4)_2SO_4$, 0.5% K_2HPO_4 and tap water, in orbital shaking incubator (160 rpm) at room temperature. Xylanase enzyme assay was carried out using 1% 4-*o*-methylglucuronoxylan from birch wood (7,500 Roth Germany) as a substrate in 50 mM sodium citrate buffer, pH 5.4 and temperature 40°C (ref. 12). Among the various fungi screened for xylanase activity, a wild strain of *Trichoderma* sp. (TNPL-192) was found to produce more enzyme when compared to others and the same was used for further studies.

To improve the xylanase production all the media components and conditions such as pH and temperature were optimized using Graeco-Latin technique. Various less expensive and easily available ligno cellulose substrates were tried and it was found that corn cobs, as carbon source, could induce substantial amount of xylanase production by *Trichoderma* sp. Xylanase production was scaled up in 5 l (operating volume 3.7 l) laboratory fermenter (Bioengineering Switzerland) using optimized medium (Table 1). The initial pH of the medium was 6.5 and allowed to vary during fermentation period. Temperature and PO_2 were maintained at 28°C and 8.0% saturation respectively. After 6 days of cultivation the crude culture filtrate had a xylanase enzyme activity of 108 XU/ml.

Crude xylanase enzyme produced in the fermenter was used for pretreatment of mill-made hard wood kraft pulp (kappa no. 17.8; brightness 24.4% ISO; viscosity 12.6 cps) prior to chlorination stage in CEH bleaching (C: chlorination, E: extraction and H: hypochlorite). The enzyme pretreated pulp along with control was bleached in the laboratory under identical conditions. The pulp properties such as kappa number and viscosity were performed as per TAPPI test methods T 236 and T230 respectively and brightness using Elrepho 2000 as per ISO 2470.

The bleaching results including bleaching conditions are presented in Table 2. From the results it is clear that xylanase enzyme pretreatment improves the bleaching response of hard wood pulp. Overall there is 4.4% ISO brightness increase over the control and

Table 2. Influence of xylanase pretreatment on bleachability of hard wood kraft pulp

Particulars	Control		Enzyme treated	
Enzyme stage (X)				
Xylanase enzyme applied XU/g	–		10	
pH	6.0		6.0	
Chlorination stage (C)				
Chlorine as Cl_2 % applied	3.20		3.20	
Consumed	3.13		3.06	
Final pH	2.2		2.1	
Extraction stage (E)				
Alkali as NaOH % applied	2.00		2.00	
Consumed	1.42		1.26	
pH Initial	12.0		12.2	
Final	11.5		11.6	
Hypo stage (H)				
Hypo as Cl_2 % applied	1.92		1.92	
consumed	1.38		1.70	
pH Initial	9.8		10.2	
Final	8.6		8.7	
Final brightness % ISO	77.3		81.7	
Brightness gain	–		4.4	
Viscosity cps	5.4		5.0	
Bleaching conditions:				
	X	C	E	H
Temperature (°C)	50	Amb	60	40
Time (min)	120	30	60	120
Consistency (%)	10	3	8	8

enzyme pretreatment has insignificant influence on viscosity of the pulp, which indicates that it did not have much pure cellulose degrading enzymes. Generally, xylanase enzyme pretreatment is used to improve the brightness target in total chlorine free and elemental chlorine free prebleaching or to reduce the active chlorine components during the bleaching by reduced chlorine dosage to save chemicals and decrease the AOX production. In the present study we have applied the xylanase enzyme prebleaching concept to improve the final brightness of the pulp without any additional chemical charge Similar to the present study, enzyme pretreatment before the peroxide delignification/bleaching is also found to improve the final brightness of various pulps[9,13].

From the above results it is concluded that introduction of xylanase enzyme pretreatment stage, (10 XU/gram of pulp) in CEH bleaching of hard wood kraft pulp before chlorination stage would improve final brightness of pulp without any additional chemical charge, resulting in lower pollutant generation. The pulp properties are unaffected.

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Greater fertility of *Drosophila ananassae* flies possessing high number of sternopleural bristles

Drosophila ananassae, a cosmopolitan and domestic species, is of common occurrence in India. It occupies a unique status in the whole of genus *Drosophila* due to certain peculiarities in its genetic behaviour[1]. The work done on population genetics, behaviour genetics and crossing-over in *D. ananassae* by Indian workers has been reviewed by Singh[2]. We conducted investigations on quantitative genetics with respect to sternopleural bristle number in Indian *D. ananassae*[3–5] and the results have shown that there is a genetic heterogeneity in Indian populations of *D. ananassae* with substantial amount of additive genetic variation for this trait

Table 1. Results of fertility tests for different selection lines (low line – L_1 and L_2; high line – H_1 and H_2; stabilizing line – S_1 and S_2)

Lines	Total number of females tested	Number of progeny produced	Mean number of progeny per female	Average of two replicates
L_1	33	528	16.00 ± 3.24	17.66 ± 2.34
L_2	33	638	19.33 ± 3.41	
S_1	33	689	20.91 ± 3.93	20.33 ± 2.21
S_2	34	671	19.74 ± 2.17	
H_1	35	1326	37.89 ± 4.55	37.33 ± 2.97
H_2	35	1287	36.77 ± 3.89	

Table 2. Comparison of mean number of progeny/female between different selection lines by means of *t*-test

Selection lines	Mean number of progeny/female	*t*-value	df	*P*
High line	37.33 ± 2.97	5.156	134	< 0.001*
Low line	17.66 ± 2.34			
High line	37.33 ± 2.97	4.564	135	< 0.001*
Stabilizing line	20.33 ± 2.21			
Low line	17.66 ± 2.34	0.820	131	> 0.05
Stabilizing line	20.33 ± 2.21			

*Significant.

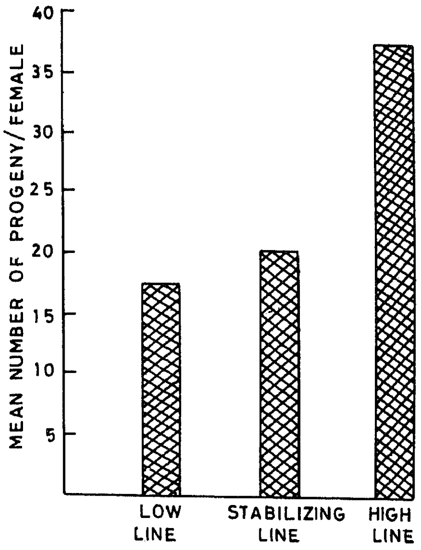

Figure 1. Mean number of progeny produced per female in low, stabilizing and high lines in *Drosophila ananassae*.

which is under polygenic control. There is also a positive response to directional and stabilizing selection for sternopleural bristle number which lends further support for polygenic basis of sternopleural bristle phenotypes in *D. ananassae*[4,5].

We conducted artificial selection experiments for high and low number of sternopleural bristles for 13 generations in *D. ananassae*. Separate high (H_1, H_2) and low (L_1, L_2) lines could be established[5]. We also conducted stabilizing selection experiments for bristle number for 15 generations and two replicates (S_1, S_2) of stabilizing selection line with intermediate bristle number could be established[4]. Mating success of *D. ananassae* flies possessing high and low number of sternopleural bristles was studied by direct observation in mating chamber and the results have shown that the females and males with high number of sternopleural bristles are more successful in mating than those with low number of bristles[6]. In order to test the effect of sternopleural bristle phenotypes on fitness in *D. ananassae*, fertility tests were carried out with flies having high (H_1, H_2), low (L_1, L_2) and intermediate number (S_1, S_2) of sternopleural bristles and the results are reported here.

Flies used in fertility tests were taken from two replicates of high line, two replicates of low line (after G_{13} of directional selection) and two replicates of stabilizing line (after G_{15} of stabilizing selection). From each line, virgin flies were collected and aged for about seven days. For pair mating, flies having known bristle number were taken from each line (in high line, females and males having more than 23 bristles were selected, in low line, females and males possessing less than 14 bristles were selected and in the case of stabilizing line, females and males having 17 bristles were selected). Each pair was placed in a fresh food vial for two days in order to mate. They were transferred into fresh food vials after mating. Three days later they were transferred to another set of food vials and again after three days, the flies were transferred to fresh food vials. Three more days afterwards, the flies were discarded. Thus there were three sets of vials (total time 9 days) for counting the progeny. The number of flies emerged in each vial was counted and the total number of progeny produced by a single female in 9 days was calculated. Mean number of progeny per female was calculated for low, high and stabilizing selection lines and *t*-test was performed to compare the fertility of flies in different selection lines.

A total of six lines were tested for fertility and the results are given in Table 1. The total number of females tested in different lines varies from 33 to 35. The mean number of progeny produced per female in different lines varies from 16.00 (L_1) to 37.89 (H_1). The average of two replicates shows that the flies having higher number of bristles produced more progeny than the flies having lower number of bristles. However, the mean number of progeny per female in stabilizing selection line is less than the high line but more than the low line. Figure 1 shows the mean number of progeny produced per female in high, low and stabilizing selection lines.

A comparison of mean number of progeny produced per female between different lines was done by means of *t*-test (Table 2). It is evident that the difference is significant ($P < 0.001$) between high and low lines and also between high and stabilizing lines ($P < 0.001$). However, the difference in mean number of progeny between low and stabilizing lines is not significant ($P > 0.05$). These results indicate that the flies having more bristles have greater fertility than those with low and intermediate number of bristles. Although the difference between low and stabilizing lines is not significant statistically, the flies from stabilizing selection line produce more progeny and show higher fertility than the low line flies.

It has been found that *D. ananassae* flies possessing high number of sternopleural bristles are more successful in mating than those with low number of bristles[6]. Thus *D. ananassae* flies with high number of sternopleural bristles are more successful in mating and also show greater fertility than the flies with low number of sternopleural bristles. This provides evidence for a positive correlation between sternopleural bristle number, mating propensity and fertility in *D. ananassae*.

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Chronic arsenic toxicity in West Bengal

I have read with interest the paper 'Arsenic in ground water in seven districts of West Bengal, India – The biggest arsenic calamity in the world', by Mandal *et al.* (*Curr. Sci.*, 1996, **70**, 976–986). Without undermining the gravity of arsenic calamity in West Bengal, I believe that many figures presented in the paper are not based on materials and methods used for the scientific presentation. Materials investigated in the study are water samples of the affected villages and hair, nail, skin and urine samples of some of the people residing in those villages. No scientific epidemiological methodology was described in the paper. Under the circumstances, how was the figure of 20% of the people suffering from chronic arsenic toxicity due to drinking arsenic contaminated water arrived at? Similarly the statement that more than 200,000 people have skin lesions is conjectural. The figure may be much less or much more. Unless an epidemiological survey using statistically designed sampling method involving the whole affected region is carried out one cannot be sure regarding the total number of the affected people.

It needs to be emphasized that diagnosis of chronic arsenic toxicity on the basis of the only finding of diffuse melanosis is fraught with over diagnosis of dark complexioned rural population as suffering from the clinical disease. One is therefore not sure whether 60% of children of Madanpur village of Murshidabad are actually suffering from chronic arsenicosis.

It needs to be further stated that muscle biopsy is not done for the confirmation of diagnosis of chronic arsenic toxicity. Though detection of high level of arsenic in body tissues like nail, hair, skin scale in association of characteristic clinical manifestations of chronic arsenic toxicity confirm the diagnosis of arsenicosis, one can have definitive features of the disease in absence of elevation of arsenic level in those tissues.

We have recently completed an epidemiological survey of South 24 Parganas, one of the affected districts with financial assistance from Rajiv Gandhi National Drinking Water Mission.

We have observed typical raindrop pigmentation of the skin and thickening of the palm and soles characteristic of chronic arsenicosis in 8.82% (152) and 3.64% (368) respectively among 4171 persons who were drinking arsenic-contaminated water (0.05–3.2 mg/l). On the other hand, pigmentation was found in 13 cases and thickening of palm and sole in 4 cases out of 3235 persons drinking water having arsenic level between 0.01 and 0.05 mg/l. None of the 277 people drinking water having arsenic level less than 0.01 mg/l had any pigmentation of skin or keratosis.

Since 1983 we have investigated 248 patients suffering from chronic arsenic toxicity due to drinking arsenic-contaminated water and attending our Post Graduate Medical Institute. The common presenting features found in these patients were rain-drop pigmentation (94.35%), thickening of palm and sole (65.3%), dyspepsia (65%), cough with or without expectoration (62.5%) and burning sensation of the eyes (29.8%). Important physical signs were anaemia (43.9%), hepatomegaly (76.6%), splenomegaly (29.4%), rhonchi and crepitations of the lung due to restrictive and/or obstructive lung disease (30.24%) and polyneuropathy (8.04%). Skin cancer was found in 2% of the case.

The clinical manifestations of chronic arsenicosis as observed in West Bengal are varied and more severe than those observed in other parts of the world.

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Mandal et al. reply:

We appreciate Guha Mazumder's comments but some clarifications are necessary.

For the last 2 years the School of Environmental Studies (SOES) is studying in detail *only one block* out of 55 arsenic affected blocks of 7 districts. The study is made on the anticipation that the results of a detailed study in one block may permit extrapolation of the situation of other blocks. Some blocks according to our survey report are known as seriously affected, like Domkal in Murshidabad, Kaliachalk in Malda and some blocks not so much affected as Baruipur and Sonarpur in South 24-Parganas and moderately affected are some blocks as Basirhat I, Deganga of North 24-Parganas. We have chosen Deganga block for a detailed study. Table 1 summarizes a detailed survey report of Deganga block for a two-year period.

Table 1. Survey report of Deganga block under North 24-Parganas*

Parameters studied	
Total area of Deganga	201.05 sq. km
Total population of Deganga	234142
Total no. of Gram Panchate (G.P.) in Deganga	14
Total no. of G.P. where arsenic in water above 0.05 mg/l	14
No. of G.P. where arsenic patients were detected	10
Total no. of Mouzas	108
Total no. of Mouzas where arsenic in tubewell water more than 0.05 mg/l	93
Total no. of tubewells in Deganga (almost)	15000
Total no. of tubewells analysed for arsenic	8500
% of the tubewells having arsenic within 0.01–0.049 mg/l	18%
% of the tubewells having arsenic above 0.05 mg/l	40%
Total shallow tubewells in use for agriculture (recently started this work)	110
% of the shallow tubewells having arsenic above 0.05 mg/l	43%
Total no. of school tubewells analysed for arsenic	84
% of the school tubewells having arsenic above 0.05 mg/l	50%
Total no. urine samples analysed	4867
% of urine samples having arsenic above permissible limit (40 µg/1.5 l/day)	86%
Total no. of nail samples analysed	1450
% of nail samples having arsenic above permissible limit (1.08 mg/kg)	78%
Total no. of hair samples analysed	1360
% of hair samples having arsenic above permissible limit (1 mg/kg)	48%

* For the last 2 years seven persons are engaged six days a week for survey work in Deganga.

Table 2. Analytical report of water, hair, nails and urine of South 24-Parganas district*

Sample	Total no. of samples analysed	Total no. of samples having arsenic above maximum permissible limit	Percentage of samples having arsenic content above maximum permissible limit	Range from above maximum permissible limit
Water	458	249 (above 0.05 mg/l)	54.37	0.05–2.22 mg/l
Urine	467	232 (above 40 µg/1.5 l/day)	49.68	40.00–9375 µg/1.5 l/day
Hair	509	274 (above 1 mg/kg)	53.83	1.00–7.43 mg/kg
Nail	464	246 (above 1.08 mg/kg)	53.02	1.08–57.4 mg/kg

Maximum permissible limit of arsenic in drinking water : 0.05 mg/l.
Normal range of arsenic in hair : 0.08–0.25 mg/kg.
Normal range of arsenic in nail : 0.43–1.08 mg/kg.
Normal range of arsenic in urine : 5–40 µg/1.5 l/day.
* These are the results of a joint project of School of Environmental Studies with D. N. Guha Mazumder in epidemiological study of South 24-Parganas.

It is clear that 234,142 people were consuming tubewell water from 15,000 tubewells (our survey reveals that most of the people drink tubewell water). On an average, 40% of the total tubewells we have surveyed (8500) contain arsenic above 0.05 mg/l, leading to the estimate of 93,600 people in Deganga block alone drinking arsenic-contaminated water. Assuming conservatively only 50% of the value we have calculated is correct, then even 2.55 million people were drinking arsenic contaminated water above 0.05 mg/l in 55 blocks.

Chakraborti and Saha[1] studied the incidence of arsenical dermatosis in 14 villages of West Bengal in 1987. Dermatosis was characterized by diffuse and palmoplanter melanosis and keratosis. According to their study the lowest arsenic concentration in water producing dermatosis was found to be 0.2 mg/l. In our study at Deganga block, about 7.3% tubewells have arsenic concentration above 0.2 mg/l. So, at Deganga block about 17,000 people are drinking arsenic-contaminated water and according to the study of Chakraborti and Saha these people, i.e. about 18% of the people drinking arsenic-contaminated water may have dermatological symptoms. We mentioned at the very beginning that Deganga is a moderately-affected block in seven districts. So our observation of about 20% of the people showing arsenical skin lesions out of the total people drinking arsenic-contaminated water in West Bengal may not be ruled out.

In West Bengal the affected villagers are farmers and they consume a high amount of water (5–6 l per day). Several studies all over the world provide quantitative toxicity data in human following chronic oral exposure to arsenic. Taking together, these studies clearly indicate that chronic intakes of about 10 µg/kg/day or higher may result in dermatological and other signs of arsenical toxicity[2]. The study of Chakraborti and Saha identified a Lowest Observed – Adverse – Effect-Level (LOAEL) of 18 µg As/kg/day of dermal and hepatic effects. This value is strongly supported by several other studies that identified similar LOAEL values: Hindmarsh *et al.*[3] LOAEL = 19 µg/kg/day; Cerbrian *et al.*[4] LOAEL = 14 µg/kg/day; Abernathy *et al.*[5] LOAEL = 14 µg/kg/day.

Table 4 of our published paper in *Current Science* reports that approximately 20% of the population exposed to arsenic-contaminated water in Taiwan, Chile, Argentina, Mexico, Thailand have arsenical skin lesions. This is similar to our prediction that 20% of the total population drinking arsenic-contaminated water above 0.05 mg/l (1.1 million) may have arsenical skin lesions. Also our survey in many villages shows that up to 40% of the people have arsenical skin lesions. In one such village (Shanpukur, Deganga) D. N. Guha Mazumder went with us and we found that 40 people out of 60 we surveyed had arsenical skin manifestation. The dermatological study carried out in Deganga over the last 2 years will be published soon.

Further, D. N. Guha Mazumder reports that recently he has completed an epidemiological survey of South 24-Parganas, one of the affected districts with financial support from Rajiv Gandhi National Drinking Water Mission (RGNDWM). In fact, one of us (DC) is a co-investigator on this project and all water (458), hair (509), nails (464),

Table 3. Arsenic content in hair and nail and dermatological features of a group of children up to age 10 years in Madanpur village, Murshidabad district, Block: Bhagawangola-II

Patient no.	Age (year)	Sex	Arsenic content in nail (mg/kg)	Arsenic content in hair (mg/kg)	P.P. Melanosis		P.P. Keratosis	
					Diffuse	Spotted	Diffuse	Spotted
1	4+	F	8.63	1.26	+	+	–	–
2	5+	F	6.52	1.27	+	+	–	–
3	5+	M	9.32	1.64	+	+	–	–
4	6+	F	17.68	3.68	++	++	–	–
5	6+	M	28.36	3.21	++	+	–	–
6	6+	M	12.35	2.36	+	++	–	–
7	6+	F	6.69	1.52	+	+	–	–
8	6	M	8.64	1.65	+	+	–	–
9	6	M	10.81	2.12	+	+	–	–
10	6+	M	4.43	1.82	+	+	–	–
11	7+	M	8.80	1.74	+	+	–	–
12	7	M	10.65	2.12	+	+	–	–
13	7+	M	25.37	2.96	++	+	–	–
14	8+	F	18.12	3.60	+	++	+	–
15	8	F	13.69	2.88	++	++	+	–
16	8+	M	4.00	1.54	+	+	–	–
17	8	M	7.91	1.88	+	+	–	–
18	9+	M	57.70	2.27	++	++	+	–
19	9+	F	7.13	1.63	+	+	–	–
20	9+	M	6.86	2.86	+	+	–	–
21	10	M	NA	2.01	+	+	+	+
22	10	M	12.88	NA	++	++	+	+

NA – Not available.

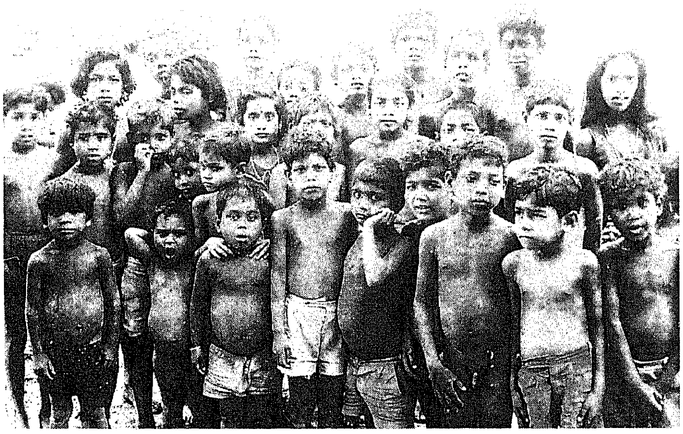

Figure 1.

urine (467) were analysed in our laboratory. Table 2 shows the water, hair, nail, urine analysis report of an epidemiological survey in South 24-Parganas. A large population who have no skin manifestation but high arsenic in hair nails, urine may be subclinically affected. The report of Guha Mazumder to RGNDWM states that, he has identified arsenical skin lesions in some people drinking water containing arsenic in the range 0.01 to 0.05 mg/l.

Although the WHO recommended value in drinking water is 0.01 mg/l, many countries including India believe 0.05 mg/l is the safe limit. Thus according to Guha Mazumder even if people get arsenical skin lesions drinking arsenic-contaminated water from 0.01 to 0.05 mg/l then the magnitude may be much higher than we anticipated.

In response to the comment 'Findings of diffuse melanosis is fraught with over-diagnosis of dark complexioned rural population as suffering from the clinical disease', we like to state that due to over length of the paper we could not provide the details of analysis of hair, nail and dermatological symptoms of the Madanpur village children. Table 3 gives the arsenic in hair, nail and dermatological features of some of the children and Figure 1 is the children of Madanpur village having arsenical melanosis (60%).

The following points will further explain the relation of dermatological symptoms and arsenic toxicity.

Pigmentations and arsenical toxicity

In the art of diagnosis of a disease, history is of prime importance followed by clinical examination findings which have to be confirmed by laboratory and other investigations. Findings of one clinical sign without correlation of the other two (history and investigations) never points to the disease. There are

Table 4. Area-wise family distribution of arsenical dermatosis

Village	District	No. of families	Population	No. of the affected family	Percentage	Total members of the affected family	No. of the affected persons	% of affection	
								In village	In family
Gangapur, Barasat	North 24-Parganas	68	243	11	16.18	55	23	9.46	41.82
Ramnagar	South 24-Parganas	24	248	10	41.70	63	58	23.00	92.10
Daulatpur, Habra	North 24-Parganas	21	119	13	61.90	79	40	33.61	50.63
Kachua, Binimoypara, Habra	North 24-Parganas	35	148	22	62.90	116	75	50.70	64.70
Kalobari, Habra	North 24-Parganas	39	168	17	43.59	101	36	21.43	35.64
Madra	Bardhaman	12	763	3	25.00	14	13	17.10	92.86
Charmajdia	Nadia	15	83	3	20.00	35	18	21.69	51.43
Gopalnagar, Karimpur	Nadia	7	51	7	100.00	51	44	86.27	86.27
Katlamari,	Murshidabad	48	335	44	91.67	317	175	52.24	55.20
9	5	270	1471	104	49.63	831	482	35.77	58.00

too many causes of isolated melanosis (diffuse or spotted) as well as isolated keratosis (diffuse or spotted). A clinician should never call racial pigmentation (i.e. Africans), dark coloured persons, solar melanosis, drug-induced melanosis, haemochromatosis, suprarenal deficiency (Addisson's disease), ochronosis, xeroderma pigmentosa and many other hyperpigmentary conditions as arsenical melanosis, because there are distinctive diagnostic criteria of each condition. The differentiating features of arsenical melanosis from all other hyperpigmentary states are taught to the students of dermatology.

When both melanosis and keratosis are present in the same patient, the problem of diagnosis becomes simplified pointing to arsenical dermatosis but these features are not present during birth or before 2 years.

As the keratin layer of palms and soles is thick compared to that of soft skin of body, palms and soles remain non-dark in dark skinned persons which becomes dark in some pathological melanotic states, easily recognized by comparing with the palms of unaffected dark persons.

In Mandapur village arsenical melanosis was pointed to the acquired darkening of palms, not from general pigmentation alone. These were confirmed by high arsenic in nails and hair (Table 3).

Arsenical melanosis is an acquired condition contrary to racial, genetic and other congenital pigmentations.

Clinical expression

From the analysis of arsenical dermatosis in 9 villages in 1985 by K. C. Saha (Table 4) it can be easily seen that all the individuals of the affected family do not have clinical signs of the disease though many of them later on developed the clinical disease if the intake of contaminated water was continued.

Many of these apparent normal subjects show high arsenic in nails, hair and skin scales proving their subclinical arsenical toxicity.

In my grouping of the stages of arsenicosis, the different stages of clinical expression have been clearly mentioned e.g. (i) Chemical or transient phase, (ii) Subclinical or persisting phase, (iii) Clinical melanosis phase, (iv) Clinical melanokeratotic phase, (v) Phase of internal complications (liver, muscles and other organs), (vi) Carcinoma.

Though arsenicosis is a generalized disease, clinical expression in skin appears earlier than the complicating phase of internal toxicity.

Muscle biopsy

For the diagnosis of arsenical toxicity, easy available tissues like skin scales, nails and hair and skin biopsy are recommended rather than routine muscle biopsy, liver or kidney biopsy.

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HEP research in the Indian context

I wish to make a few comments on the special section on high energy physics (*Curr. Sci.*, 1996, **71**, 109–127) in this note. G. Rajasekaran says that new ideas for particle accelerators have to be discovered or there will be an end to HEP by about 2010 AD. Is Indian science ready to entertain new ideas? The idea to discuss HEP in the 21st century itself is not new; it is a borrowed one. Ramachandran lists outstanding questions for the early 21st century suggested by Gross, Witten and Kane. There is nothing wrong in relying upon experts, but the country's science leadership almost always follows the frontiers set in the West. It is unfortunate, but true, that the research environment in our country is so sterile and conformist that an original mind has to opt out of the system to sustain creativity. I doubt, if C. J. Joshi whose leading contribution in the plasma beat wave accelerator has been noted by A. Sen, would have got necessary support had he been in India.

Idea-wise, I think alternatives to the experimental philosophy embodied in the 1911 Rutherford scattering experiment might be worth looking at. After all, the aim of HEP is to search for a fundamental entity and foundational theory; and for that maybe going to higher and higher energies doing Rutherford scattering-like experiment is not the right choice. A plethora of elementary particles could be just the energy clumps created due to accelerating fields, having no fundamental significance. Why cannot a few massless particles be the building blocks of matter? Though neutrino oscillation and finite non-zero mass are of current interest in view of the unresolved solar neutrino problem, a neat theory with massless neutrinos could be envisaged for composite structure of photon and this together with the electron, might be the basic constituents of matter. De Broglie did consider this hypothesis, however, at that time muon neutrino was not known and the objections to this idea essentially originated from quantum theory, and the massive photon predicted in this theory conflicted with massless photon. Recently the possibility of topological quantization as an alternative to quantum theory has been explored, and some significant advances in the knot theory made. In particular, the discovery of the Jones polynomial in 1984 which can distinguish mirror image of a knot, and Witten's physical interpretation given in 1988 using 2+1 dimensional field theory are important for the present discussion. One might revive the idea that neutrino is a space–time trefoil knot in the light of these new discoveries (H. Jehle in 1975 considered a trefoil model). These ideas are at present what Rajasekaran calls the 'crazy ideas', however, such alternatives need to be given attention since the physics at Planck energy and early universe is very speculative, and the standard model has at least two major drawbacks, i.e. large number (as many as 20) of adjustable parameters and mathematical weakness of dealing with infinities in renormalization procedure.

Rajasekaran's proposal for a task force of a mutidisciplinary team of experts for new discovery in accelerators is endorsed by Ramachandran. In the most successful laboratories the world over, this approach has been in practice since long; unfortunately in India such proposals adorn the recorded speeches of eminent scientists, and are seldom implemented. Unless the problems at implementation level are sorted out, the think-tank proposal by Rajasekaran is likely to remain wishful thinking.

Ramachandran notes the criticisms voiced against heavy funding for doing science for the sake of it, and tries to justify public support for HEP, citing its impact on other branches of science and high-tech fall-outs of accelerator R&D. In 1994, the *CERN Courier* highlighted the spin off and technology transfer from HEP research. Hoffman in an article (*CERN Courier* 1994, **34**, 7–14) provided an exhaustive discussion on the subject. My question is: what is the spinoff of HEP in India? Ramachandran has not given any example from India. Unless a definite 'market value' in terms of indigenous technology development is specified, the proposal for building high energy accelerator (in the range of 10 GeV–20 GeV) shall be an avoidable burden on the public funding.

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Rajasekaran and Ramachandran reply:

S. C. Tiwari's comments appear to be somewhat obtuse, generally lamenting on the poor impact of Indian science. The special section on the contrary, is a status report on High Energy Physics in the global context, although it includes some suggestions relevant to this country. The criticism that proposals such as research on new methods of acceleration are seldom implemented in India is a valid one. The point of repeating such proposals, nevertheless, is the hope that it will stimulate some young minds to think in this direction. It may be appropriate to mention here that for the first time, a School on Accelerator Physics is going to be held in India (School on 'The Physics of Beams' at the Centre for Advanced Technology, Indore, 13–25 January 1997). Hopefully this will be the first step towards the goal of generating new ideas on particle acceleration.

Instead of pursuing the beaten track of scattering-type experiments, can one envisage alternative experimental routes to deeper regions of space–time? This is an interesting question. However, Tiwari's list of speculations on basic structure does not answer this question. They are rather vague and even if they could be made a part of a consistent theoretical structure, they also have to be confronted by experiments. What is required is a new experimental paradigm.

We shall not enter into the broader question of public support to fundamental science here. It is an important question already discussed at various fora.

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Ecological economics – Towards a synthesis of two disjunct disciplines

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The basis of all human activity is the use of natural resources, which also constitute the basic life support system. All economic activity, directly or indirectly, derives its sustenance from the environmental resources. The correlation between economic growth and natural resource depletion has of late attracted the attention of economists, but they form only a minority. The present article addresses basic issues leading to differing perceptions among ecologists and economists as far as economic growth is concerned and also tries to explore how this gap could be narrowed. The paper also reviews in brief some of the most recent international debates relating to ecological economics. The intricate problems of economic evaluation of ecosystems and the difficulties encountered in this process have been highlighted.

THOSE of us who were fortunate to have gone through the special issue of *Ecological Economics*[1] would agree how a meaningful dialogue between ecologists and economists has attempted to bridge the wide gap between the two most dominating disciplines of the twentyfirst century. This process had, however, started much earlier with the works of Costanza and Daly[2], Costanza[3], and Maxwell and Costanza[4]. The Asko meeting, organized by the Beijer International Institute of Ecological Economics in September, 1994 in the archipelago outside of Stockholm, was an important landmark in further concretization of this process of dialogue and consenus. The participants included eminent economists and ecologists and the dialogue resulted in a joint article by the distinguished scholars, led by Kenneth Arrow, which got published in *Science*[5]. However, Robert Costanza, a co-author of the Arrow *et al.* article, in his introductory essay on 'Economic growth, carrying capacity, and the environment' in the special issue[1] has outlined the reason that 'like any consensus statement, Arrow *et al.* leave many things vague and only scratch the surface of many others[6]'. It was, therefore, logical to elicit further response to the paper from a group of invited scholars, who could feel free to offer positive, negative or both responses which is not possible in a consenus group. These responses were slated to be published in three separate journals and one of them is *Ecological Economics* which is being discussed here. One of the commentators[7] in the issue pointed out that the article by Arrow *et al.* was some sort of 'dog bites man story', in the context of 'the article's authorship, novelty of the concept and the journal's prestige and readership'. That was perhaps the reason it initiated a debate and a discourse which could have academic implications on one end and possibly political on the other. However, in terms of the novelty, it does not seem to be a 'man bites dog story' as was said to be. There are indications of such a thinking in economics much earlier. Here it would be interesting to refer to an obituary essay by Herman E. Daly[8] who, while paying tribute to his distinguished teacher, Nicholas Georgescu-Roegen, mentioned that there was a great need for introspection among economists, who refuted Georgescu's revolutionary ideas not by reasoning, but rather by keeping silent or making 'the lame remark' that his recent work was 'not really economics'. The revolutionary ideas were contained in Georgescu's *Entropy Law and the Economic Process*[9] and *Analytical Economics*[10], but even after three decades standard economics textbooks have ignored these reformist ideas of Georgescu-Roegen. Daly in this essay has drawn the readers' attention to Paul Samuelson's Preface to *Analytical Economics* in which he referred to Georgescu-Roegen as a 'scholar's scholar', an 'economist's economist' and eulogized him elsewhere by saying, 'I defy any informed economist to remain complacent after meditating over this essay'. But what was preached was never practised. Samuelson's 'influential textbooks', to quote Daly, have not contained a trace of Georgescu's ideas. Why, for example, was it not possible for the concept of 'entropic flow' to occupy the core of economic theory? The 'circular flow' continued to have sway over economic policy because in this path it was theoretically possible to achieve a continuous economic growth through abstract exchange value (debt, purchasing power) which has no physical dimension[8]. For example, the circular flow diagram conveys the meaning of the economic process as an iso-

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lated circular flow from the manufacturers to the users and back again, without any inlets or outlets. In sharp contrast is the Georgescu-Roegen's view of one-way flow which begins with resources and ends with waste. He called it 'entropic flow' and highlighted its role in the maintenance of economic process. In his analysis he brought out clearly that the entropic flow necessarily induces changes in the environment on which it depends, because what is released as waste is qualitatively different from what is taken in[8]. Georgescu also explained that inclusion of physical dimension in the circular flow of economic process had not been considered at all by economists. Georgescu-Roegen used the second law of thermodynamics or the entropy law to explain the matter–energy flow in economic process by relating it with the environment in terms of resource utilization and production of waste. Consider conversion of soil (clay) into bricks which are then used for construction of a building and one can in no way retrieve soil from the concrete mass. Also add to the process amount of energy used up in converting clay into bricks. The system, therefore, ends up with taking in low entropy energy-matter and producing high entropy energy-matter. Daly argues that growth in entropic flow encounters physical barriers like depletion, pollution and ecological disruption. Since, in mainstream economics, growth in GNP has been treated as synonymous with the economic growth, it has been impossible to set limits to which resources can be exploited and converted into capital. Georgescu-Rogen considered substituting capital for resources, an idea propounded by the neoclassical school, as the cardinal sin of economics. He therefore, could not perhaps expect a better deal from his peers. So much for the 'disciplinarian' attitudes.

Ecology and economics

There is a highly striking similarity between physical sciences and economics, of course via mathematics, in achieving results through submission to experimental data and their verification by empirical evidence. Also that when 'economic phenomena are analysed thoroughly they display existence of regularities which are just as striking as those we find in physical sciences'[11]. It is this virtue of economics which has been exploited in the past and also in the present day neoclassical thinking. For instance, the theory that the resource constraints of entropic flow can be overcome by substituting capital for resources owes its origin to the Cobb-Douglas type production functions. But in reality how much substitutability is possible between fund and flow remains a moot question! Conversion of resources into capital would follow that not only more capital is required for the purpose, but also more resources are needed to generate fund flow. More production to generate capital would also result in depreciation, depletion, pollution and disruption. In the words of Daly, 'to conceive of capital as a near-perfect substitute for resources, as is frequently done under the influence of Cobb-Douglas type production functions, is to believe that one can make the same house with twice as many saws, but half the lumber. Not to mention the problem that more saws require more resources for their production[8]'. It is on this count of over-mathematization of economics and the elevation of technique over substance[12] that has, in a way, led to the wedge between ecology and economics.

Biology in general and ecology in particular, compared to other scientific disciplines, seem to exhibit lesser regularities/generalities which could be analysed and forecast. 'Ecology has deep-rooted characteristics that make its problems more difficult to solve than, for example, those of physics[13]'. Therefore, modelling exercises and projections, derived through mathematical equations, for solving ecological problems, more often than not, fall flat while dealing with the intricacies of life phenomena. Since problem solving is a difficult task in ecology, some authors have defined it as 'the most intractable legitimate science ever developed[13]'. Consider extrapolation of an observation made on one organism to all organisms inhabiting the same ecosystem. For instance, temperate forests in Alps and Himalaya harbour *Sorbus aucuparia*, cannot be generalized to mean that biotic communities of the two biomes are similar. Similarly, populations of a species, distributed in time and space, may have similar genetic make up, but differ greatly in their ecological properties. Prediction of likely habitats of these species-populations, using analytical tools, simply does not work because populations of same species may inhabit extremely contrasting habitats. A practical weakness of the approach, using regularity or generality as a basic criterion in problem solving in ecology, is that predictions generated are not sufficiently precise to answer specific questions about specific individuals and localities. Similarly, theories like sigmoid population growth equation, also known as logistic or Verhulst-Pearl equation, are 'pedagogically useful but not applicable to specific situations, because they ignore such complications as age distributions, time lags and nonlinear interactions that characterize actual populations[14,15]'. The problem is further complicated when equations and the analyses derived from these are applied to complex life-form situations like ecosystems.

One such analysis, based on equations and extrapolations, which has of late attracted great attention among economists and biologists alike, relates to environmental and natural resource accounting. A plethora of literature is now available on the subject of system of national accounts (SNA) and a review of the debate was published by UNEP in 1993 (ref. 16). In the majority economic view, the basic assumption in the whole SNA analysis revolves around the GNP as the index of growth, which in turn is based on the quantification of goods and services, and stocks and flows. There is, however, a growing realization among economists that if the welfare of future generations is to be kept in mind then the net national product (NNP) is a better index than GNP. These economists[17] are of the view that 'changes in stocks of natural resources have typically not been included in the SNA. As many of these resources generate essential life supporting services, their exclusion may severely distort the NNP estimates. It should be of highest priority to try to include changes in the complete asset base in the accounts'. Even for estimation of NNP, in the ecological context, the evaluation will embrace the natural capital, which is an ecosystem or the species therein. For estimation of NNP, it is natural that, while taking care of the ecological constraints, we must resort to shadow pricing of goods and services of an ecosystem. To achieve this sub-goal it is necessary to build demand supply curves for the various ecological functions. A number of methodologies are being attempted internationally to evaluate ecological functions in terms of services, but agreement on these seems a remote possibility, even among economists[16]. It is extremely difficult to build demand supply curves, precisely because we are unable to know how and which functions to quantify in an ecosystem. Applying economic tools of shadow pricing would involve methodologies like opportunity cost, revealed preferences, willingness to pay and accept, travel cost and many more. All these methodologies suffer from one basic flaw – they are all anthropocentric and thus ignore all real natural functions of an ecosystem. It can be argued here if there can be any other view than 'anthropocentric' in economics. At the outset it seems extremely difficult and impracticable, but in long range view, economics cannot escape from the reality that the resources, which form the feed of economy, cannot be exploited unsustainably for long. Economics has to be on the lookout for alternate development paths, which are sustainable. Carrying capacity based development and the holistic context of institutional economics are some of the useful approaches for achieving sustainability[18,19].

On a somewhat transformed note the neoclassical school believes that after all one can quantify most of the variables operating within an ecosystem and model the effect of different scenarios that could arise and, therefore, select a development path that could be termed as sustainable. As stated above, whatever be the methodology this analysis cannot escape the basic bias towards human species and the subservience of all other life to it. Let us take an example of a terrestrial ecosystem and consider whatever could be natural functions of a pristine ecosystem and then let us also find out what are human aspirations linked to this ecosystem (Figure 1).

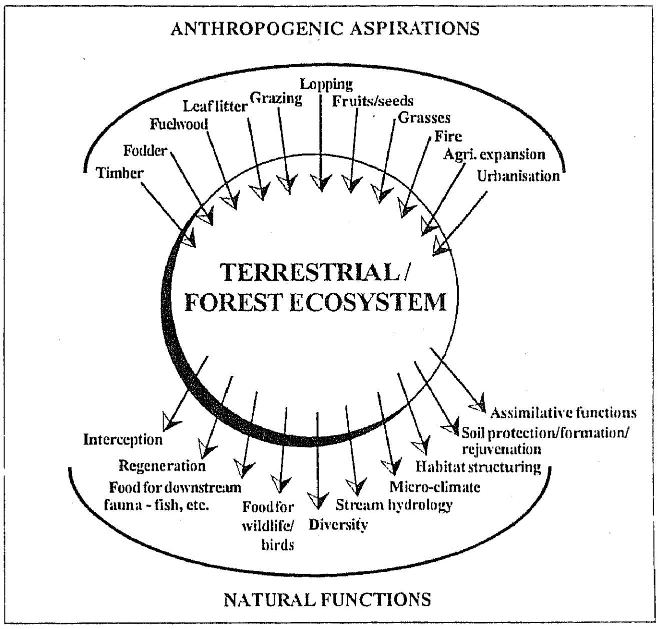

Figure 1. A simplified summary of anthropogenic pressures on a forest ecosystem and its natural functions. Note the diversity of natural ecosystem functions ranging from maintenance, regulation, habitat structuring, assimilation, etc. Any one or a mix of human activities as shown in the top of figure can severely damage or reduce the natural functions, which in turn would destroy the very base on which that human activity depends. Such natural functions, therefore, cannot be ignored while attempting natural resource accounting.

Table 1. Components included in modified forest accounts

Yield	Maintenance	Stock
Timber	Maintenance	Forest inventory
Harvest of berries		Berry-yielding herbs
Mushroom		Mycelium
Meat (bag)	Game protection	Game population
Recreation	Various activities	Various
Biodiversity	Fauna/flora protection	Survival conditions
Effects on hydrological flows	Measures that affect run off	Forest inventory, bare lands, ditches, etc.
Fixing of carbon	Silviculture and boarding (non-harvesting)	Carbon pools
Buffering of acid rain, tree nutrition	Liming, fertilization	Content of exchangeable base cations in soil and vegetation
Nitrogen leaching	Construction of nitrogen sinks	Nitrogen-fixing capacity
Reindeer forage		Lichen stocks

Source: ref. 20.

From an economic analysis point of view, accounting in a natural ecosystem is done broadly for its functions like production/yield, maintenance and stock (see Table 1). When we try to understand the natural ecosystem

Table 2. Modified NNP accounts for income from Swedish forests, 1987

Market value of timber	18.63
Inputs from other sectors	–3.14
Stock growth	3.8
Silviculture	– 1.55
Subtotal 1	17.74
Berries	0.5
Mushrooms	0.55
Meat from game	0.47
Subtotal 2	1.52
Biodiversity	– 0.6
Carbon pools	3.8
Exchangeable cations in soil	– 0.6
Lichen stocks	– 0.02
Subtotal 3	2.58
Total net income	**21.84**

Source: Adapted from ref. 20. The figures are in billion SEK.

functions as outlined in Figure 1, it would be clear how little justice we do to ecology by evaluating or accounting only that which is of direct benefit to *Homo sapiens*. The natural functions listed in the Figure 1 may again only be a miniscule of the total processes operating and products being generated within that ecosystem. The evaluation leaves no scope for assessment of countless interactive processes which operate on a time-scale varying from millionth of a second (at molecular level) to millions of years (at ecosystem level). Table 2 represents the results of an exercise some authors[20] in Sweden carried out based on the components selected for accounting as shown in Table 1. One can, almost instantly, see a dichotomy between the desired outcome and the actual outcome. It is only timber, berries, mushrooms and meat from game which are added to accounts. However, the most important part, the biodiversity, which has made all other functions possible is subtracted, as if it were a liability on the system. Timber, berries, mushrooms and meat all consumed by man, are therefore, not natural functions of an ecosystem to the extent that man has extricated himself from this ecosystem through a 'civilizational process'. His dependence on forest produce cannot be equated with that of a tiger, elephant or a reindeer. Man's relation with the forest is increasingly becoming a unidirectional process, while that of wildlife is dynamic and losses and gains flow both ways.

What is, therefore, important is the understanding that *the wood* is not timber alone. What is also important is that inventory of species, as outlined in Table 1, cannot be accounted separately; the *parts* cannot be summed up as a *whole*. A forest cannot be treated like a car whose chassis, engine, gear box, etc. can be accounted individually and then totalled into an entity called car. What needs to be appreciated is that a structure we term as an ecosystem is the product of millions of years of interactions and adjustments among its components, and its biotic and the abiotic characteristics. Therefore, evaluating only a tiny fraction of this vast organism, spread both in time and space, can only be a notional exercise. The results of such an exercise, based on half truths, are going to be grossly misleading. The argument that some data, which can be bad data, is better than no data[21] is bad in intent in the same way as bad economics cannot be better than no economics. The reason is preference, while accounting, to some data like timber or non-forest timber produce over other species of seemingly modest standing in a natural ecosystem. It leads us to a situation where it becomes terribly bad ecology. In a natural ecosystem there are no preferential treatments – the high and the lowly, the mighty and the meek, are allocated resources on an equitable basis. The evidence for this comes from the fact that in a climax natural ecosystem it is generally *K-selected* species, which co-exist with a high degree of species diversity, and leave enough resources and space for others to prosper. In sharp contrast is the situation of the highly-disturbed ecosystems where it is mostly *r-selected* species, which are weedy, allelopathic and dominate to oust others. Applying classical or neo-classical economic approaches to ecosystem analysis for resource accounting would sharply bring into focus that face of economics which will juxtapose human selfishness with the co-existing nature of ecology operating in natural ecosystems. It is here that the role of eminent and influential economists can bring about a positive transformation, which shall not be perceived as correction of current and prevalent economic thought, but its evolutionary responsibility.

Keeping in mind the long term goal of sustainable development, many seminal thinkers of this century have discussed the relation between economic growth and environmental quality, and the link between economic activity and the carrying capacity and resilience of the environment. The debate aimed at establishing a substantive dialogue among economists and ecologists for exploring the possibility of an interdisciplinary consensus on the issues of economic growth, carrying capacity and the environment is a well-directed step towards synthesis of the two disjunct disciplines[1,5]. These useful efforts have to be received with admiration, support and understanding, for that can help us in evolving a methodology that allows the coming generations of *Homo sapiens* and also our contemporary life-forms to feel secure and assured of life enjoyment as is intended in the best spirit of economic thought. It is in this context that economics can find ecology to be useful. Economics tends to assume that change takes place in a fairly continuous fashion, but change in an ecosystem may be of capricious nature resulting from the response to destabilizing factor/s rather than a process of graduated change[22]. From ecology we learn the lesson of 'holism'. In other words, if environmental responses to economic change have to be predicted, various interactions and links within and between ecosystems have to be fully fathomed. The concern – how can one dispense with the anthropocentric principle of value in economics and how can a 'holistic' state be accomplished – may be genuine. But if the aim of sustainable development has to be achieved, a realization must start in right earnest that 'ecologically bounded-possibilities of using natural resources are taken as a normative starting-point for the development of economic theory[18]'. There is a great and urgent need to strengthen the new school of thought based on an institutional framework. This framework shall neither be based on anthropocentric and close-ended mathematical models, nor on a reductionistic–mechanistic argument, but on a holistic, evolutionary, open-ended and pluralistic methodological paradigm. These reformist views[23] have started gaining acceptance among economists, though only in a limited way. If we fail in this endeavour then the existing imbalance of power in the society, which helps the 'vested economic interests to put their individual short-term aspirations ahead of the collective and long-term interests of a sustainable society', will continue to increase[18]. Our success will lie in forging towards an ecological-economics synthesis. Barbier *et al.*[24] have enunciated a number of steps to enable this synthesis. Better communication between different disciplines and development of interdisciplinary approach have been suggested to be the first step towards this goal followed by improved research collaboration between organizations and individuals. This aim could be an achievable one. After all there are many common grounds of meeting in ecology and economics – equity, resource allocation, micro-economics (welfare of individuals/species), macro-economics (welfare of populations/communities/ecosystems/biosphere), stocks, flows, goods, services, etc. These are not merely semantic similarities, but real scientific regularities shared by the two disciplines. Ecology and economics are not such aliens to each other as many sceptics may think.

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A glimpse into the fascinating world of fullerenes

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Fullerenes, carbon nanotubes and onions have caught the imagination of the entire scientific community. The interest seems to be growing unabated; about two papers per day are being published today. This article sketches the fascinating area of this new allotrope of carbon, maybe the only allotrope of any element discovered in 20th century, focusing primarily on C_{60}. The early history, synthesis and characterization, mass spectrometry, derivatization, orientational ordering, pressure effects, superconductivity, magnetism and photophysical properties of fullerenes and fullerene-based compounds will be discussed. The science of buckminsterfullerene, and fullerenes in general, is still dominated by spectroscopy and chemical physics. Although many applications were proposed in the early days of fullerenes, many of them turned out to be disappointments. However, potential uses of fullerene-based materials in photophysical, biological and chemical applications are increasingly recognized.

THE role of serendipity in scientific discoveries is widely recognized. The story of buckminsterfullerene[1], C_{60} and fullerenes in general, is no exception. Scientists in their fascination to understand the chemistry of interstellar molecules, hit upon an unusual and most extraordinary discovery in the history of chemistry. The molecule they discovered had sixty equivalent carbon atoms which formed the pattern of a football giving it the highest symmetry. Theoretical predictions[2] of such structures were known from 1970. There is no molecule formed by the same atom in chemistry as big as buckminsterfullerene. Elemental carbon has been known for millennia to be occurring in two polymorphic forms, graphite and diamond. Graphite has two dimensional layers of sp^2 hybridized carbon atoms interlinked by weak van der Waals forces. Since the inter-layer interaction is weak, graphite is used as a lubricant. Diamond, however, is one of the hardest materials known to man. This property arises from the strong three-dimensional bonding in diamond in which each sp^3 hybridized carbon atom is bonded to four other similar atoms. Fullerenes constitute another allotrope of carbon, probably the first allotrope of any element discovered in recent times. Figure 1 shows the structures of graphite, diamond and C_{60}. There are also other all-carbon molecules similar to C_{60} with cage structure, collectively called fullerenes in honour of the famous American architect Buckminster Fuller whose geodesic domes are landmarks of 20th century architecture. There are other forms of carbon such as carbon rings[3] which are receiving considerable attention today.

The 1996 Nobel Prize in Chemistry was awarded to Sir Harold Kroto of University of Sussex, UK and Robert Curl and Richard Smalley of Rice University, Houston, Texas, for their work on buckminsterfullerene.

It was only natural for a molecule of such immense beauty to attract the whole of the scientific commu-

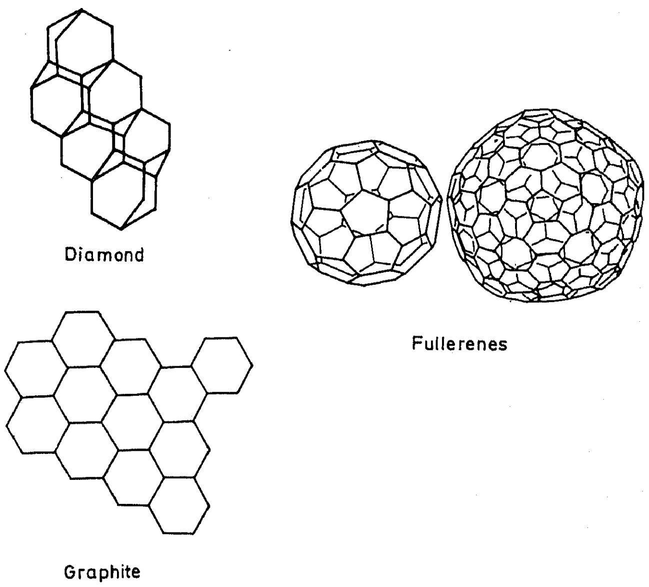

Figure 1. A schematic representation of the structures of graphite, diamond and fullerenes. While the two-dimensional sheets formed by hexagons are packed one over another in graphite, the diamond structure is three-dimensional. Only two fullerenes are shown. The smaller one is buckminsterfullerene, C_{60}. The bonds between hexagons are more like double bonds showing the corannulene-type substructure. The double bonds are localized exocyclic to the pentagons giving [5]radialene character to the pentagons and cyclohexa-1,3,5-triene character to the hexagons.

nity[4,5]. The structure, spectroscopy, chemistry, materials science and applications have been intensely investigated. Because of all these, buckminsterfullerene became the subject matter of nine out of ten of the most cited papers in chemistry in 1991. The position improved further in 1992 to make ten out of ten. According to statistics[1], one paper per week got published in this area from 1985 to 1990. After 1990, it is said do be one per day. In 1995, it was slightly less than two per day.

Molecules with 70, 76, 84 and other numbers of carbon atoms were soon characterized. Developments in 1991 showed that these molecules need not be found only with tens and hundreds of atoms but also with thousands of atoms. These giant molecules of carbon occur as nanometer size tubes and balls which are called carbon nanotubes and onions[6] respectively. A number of new geometries are proposed in recent researches[7]. The chemistry and physics of the new forms of carbon will continue to excite scientists in the coming years.

In this article, I intend to give an account of the chemistry, physics and materials science of this new form of carbon in a rather illustrative fashion. I may not present a balanced view of the subject matter as my own interests in the area are rather limited. There have been numerous conferences on fullerenes and even on specialized topics of fullerene chemistry and physics over the years. Several books[4,5] on fullerenes have appeared in the recent past and a comprehensive review in the limited space is nothing but impossible.

Discovery and early years

As noted earlier, the search for certain linear molecules of carbon normally found in the interstellar region called cyanopolyynes, was the starting point of this search[8]. Some of these molecules of the type H–C≡C–C≡C–C≡N or HC_5N have been synthesized in the laboratory. These molecules containing seven, nine and eleven carbons, were detected by radio-astronomy. However, studies of carbon arc have shown the presence of molecules containing as many as 33 carbon atoms. This was the time when Smalley and co-workers in Houston were working with a newly-developed cluster source[9] which used lasers for evaporation, supersonic molecular beam expansion for clustering and photoionization mass spectrometry for detecting the products. Similar studies were also done by Rohlfing *et al.*[10] in Exxon[10]. Their studies on graphite which preceded those of Smalley and colleagues showed the presence of carbon clusters heavier than C_{33}, but interestingly no odd number cluster was seen. Soon after the beginning of these measurements, it was observed that species such as HC_7N and HC_9N are formed in the reaction of C_n ($n < 30$) with H_2 and N_2. However, the major discovery turned out to be not the detection of cyanpolyynes but the unsually abundant species C_{60}, which dominated the mass spectrum under certain clustering conditions[11]. There were other heavier clusters too. It was found that these clusters were particularly unreactive compared to the lower clusters. Reactivity and photofragmentation studies showed that the 60 atom cluster is extremely stable. The observed chemistry could be explained if one assumes the graphitic sheet to transform into a hollow chicken-wire cage similar to the domes of Buckminster Fuller[12]. Such a closed cage requires that[13] $12 = 3n_3 + 2n_4 + 1n_5 + 0n_6 - 1n_7 - 2n_8 - \ldots$, where n_k is the number of k-sided faces. For carbon, the only values of k are 5 and 6, although 7 is also possible as has been detected recently in carbon nanotubes[14]. This means that there should be 12 pentagonal faces and the number of hexagonal faces are arbitrary. In C_{60}, there are 12 pentagonal faces and 20 hexagonal faces. Fullerenes, the class of closed cage molecules, have the general formula C_{20+2n6}. C_{60} has only one chemically distinct carbon atom. However, in C_{70} there are five distinct carbon atoms. In larger fullerenes there is the possibility of isomers and some fullerenes are also chiral. Very recent studies have shown that there are many isomers to some fullerenes than previously thought of[15]. However, because of the isolated pentagon rule (observed generally), which places pentagons separated by hexagons, the number of isomers is limited.

The experimental apparatus used in the discovery of C_{60} is shown in Figure 2. This set-up is now commercially available for the study of a variety of clusters. A rotating disk of graphite is irradiated with a powerful laser to evaporate carbon. As the laser falls on the disk, a stream of helium gas is passed over the disk by releasing a valve. The gas carries the evaporated carbon species with it and during its passage to the nozzle, the species in the vapour undergo clustering. The cluster

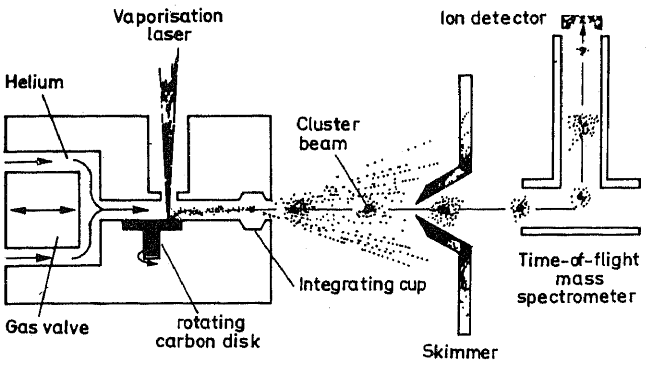

Figure 2. The experimental set-up used to discover C_{60}. The graphite disk is evaporated with a NdYAG laser and the evaporated carbon plasma is cooled by a stream of helium coming from a pulsed valve. The clusters of carbon are produced in the integration cup and are expanded into vacuum. The ions are detected by time of flight mass spectrometry (from ref. 1).

Box 1. Synthesis and purification of fullerenes

For the synthesis of fullerenes all that you need is a welding transformer, a chamber connected to a vacuum pump (even a single stage oil sealed rotary pump is adequate) and some graphite rods. The graphite electrodes are brought in close contact with each other and an arc is struck in an atmosphere of 100–200 Torr of helium or argon. To sustain the arc, a voltage of 20 V (AC or DC) may be necessary. For a graphite rod 6 mm in diameter, about 50–200 A current may be consumed. Generally, spectroscopic pure graphite of high porosity is used so that evaporation rate is high. The soot generated is collected on water-cooled surfaces which could even be the inner walls of the vacuum chamber. After sustaining the arc for several minutes, the vacuum is broken and the soot is collected and soxhlet extracted for about 5–6 hours in toluene or benzene resulting in a dark reddish-brown solution which is a mixture of fullerenes. 20–30% of the soot so collected is soluble, this soluble material is subjected to chromatographic separation[107]. Over the years, several simple methods have been discovered including the filtration technique over an activated charcoal–silica gel column[108]. About 80% of the soluble material is C_{60}, which can be collected in one pass using toluene as the mobile phase. C_{70} can be separated using toluene/*o*-dichlorobenzene mixture as eluant. Repeated chromatography may be necessary to get pure C_{70}. C_{60} solution is violet in colour while that of C_{70} is reddish-brown. Higher fullerenes C_{76}, C_{78}, C_{82}, etc. require HPLC for purification[109]. Spectroscopic properties of several of these fullerenes are now known. Normally, preparation of a gram of C_{60} requires about 5 hours of work. But many of the higher fullerenes require over 250 hours to make about 1 mg. C_{60} and C_{70} are now commercially available from several sources. C_{60} is about $175 a gram but C_{70} is not yet affordable for synthetic chemists. Therefore, a majority of the studies reported is on C_{60}. Fullerenes crystallized from saturated solutions retain solvent molecules and removing them may require long hours of vacuum drying. Crystal growth by vapour transport is an excellent method to grow millimeter size crystals devoid of solvent for sensitive measurements. For solid state spectroscopic measurements, it is better to use evaporated fullerene films in high or ultra high vacuum to avoid solvent contamination. Evaporation is also used as a method of purification as there is substantial difference in the onset of evaporation between C_{60} and C_{70}. Calixarenes (bowl-shaped macrocycles with hydrophobic cavities) have been used recently[110] in the purification of fullerenes. See Figure 4 for a schematic procedure of synthesis and purification.

Arc evaporation is not the unique way of making C_{60}. Fullerenes have been found in flames[111], upon chemical vapour deposition used to produce diamond[112], in a 1.85 billion year old bolide impact crater[113] as well as from spacecrafts[114]. It has also been made from diamond[115]. No one has made it by chemical reactions but such a possibility has excited many organic chemists[116]. It has been synthesized from camphor recently[117]. Mass spectrometry has shown that higher clusters of carbons could be formed by laser evaporation of polymers[118]. Highly unsaturated carbonaceous ring systems upon laser evaporation[119] produce C_{60}. There are several other exotic means of producing C_{60}. However, the total synthesis would indeed be a landmark in chemistry; approaches to this have been suggested[120].

Synthesis and purification was followed by the characterization of this material by a variety of spectroscopic techniques. The pivotal role played by mass spectrometry in characterizing fullerenes cannot be overemphasized. NMR showing a single line corresponding to the equivalence of the carbon atoms[22], single crystal X-ray structure resolving the atomic positions[121], characterization by UV/VIS (refs 107, 122), IR and Raman[123] spectroscopies, etc. soon followed. Predicted electronic structure was confirmed by HeI and HeII photoelectron spectroscopies[124].

beam emanating from the nozzle is selected by a skimmer. The clusters are then subjected to mass analysis by time of flight mass spectrometry. Under certain experimental conditions, the mass spectrum was very similar to that reported previously, showing a distribution of even-numbered species. But a variation in the experimental conditions, especially the introduction of the integration cup (see Figure 2), increased the intensity of the sixty atom cluster to such a point that in some experiments only C_{60} and C_{70} were seen (Figure 3).

At the same time, experiments were also done using a Fourier transform ion-cyclotron resonance (FT-ICR) apparatus. In this set of experiments, mass selected cluster ions were subjected to reactions with a variety of gases. C_{60}^+ was extremely unreactive to gases such as O_2, NH_3 and NO. On the contrary, clusters of other elements such as Si showed high reactivity. In fact no evidence was there to show that Si_{60}^+ was special. A body of other experimental data was accumulated by the Houston group on the photophysics, photodetachment

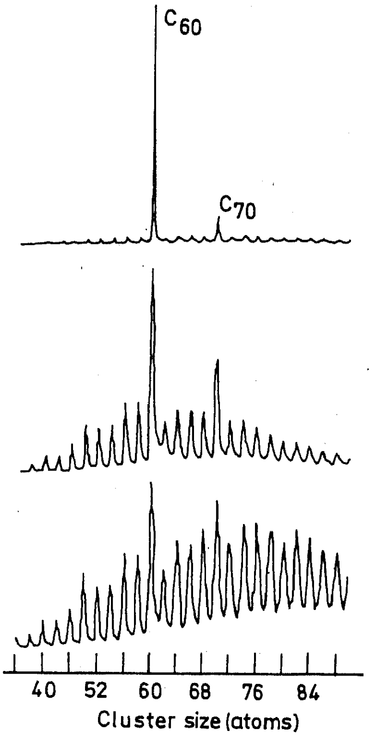

Figure 3. The mass spectrum of the carbon clusters under various experimental conditions. Under certain conditions, only C_{60} and C_{70} are seen (from ref. 1).

and optical spectrum of C_{60}. None of these studies gave any evidence to suspect the proposed structure.

For nearly five years, C_{60} was truly a chemical physicists playground. A large number of theoretical papers got published on the electronic structure[16], reactivity[17], magnetism[18] and a number of other properties of C_{60}. The studies were made easy by the very high symmetry of the proposed structure. In fact a close similarity between the observed peaks in the IR spectrum of an evaporated carbon soot and the theoretical frequencies[19] made Krätschmer and colleagues[20] look for C_{60} in the soot. They were working on laboratory-produced carbon soot in order to understand the interstellar spectrum of carbonaceous materials. The soot they obtained after evaporating graphite resistively in an atmosphere of helium contained four bands in the infrared region. The absorption frequencies correlated well with the proposed bands of C_{60}. Application of solvent extraction yielded significant quantities of fullerenes[21] from the soot and a host of techniques were applied to the characterization of this newly made form of carbon. For a description of the synthetic procedure, see Box 1.

Mass spectrometry and ion/molecule reactions

Soon after a method of macroscopic synthesis of C_{60}, several ion/molecule reaction studies were performed[22].

In the early days, fullerenes were expensive and mass spectrometer was the ideal reaction vessel. C_{60}^- and $C_{60}H^+$ were produced by chemical ionization (CI) with methane[23]. It was shown that C_{60}^- was 15 times more abundant than $C_{60}H^+$, consistent with the high electron affinity. Methane CI spectrum showed protonated fullerenes and adducts of fullerenes with C_2H_5. Collision-induced dissociation of C_{60}^+ in the keV range with helium showed the expected C_{58}^+, C_{56}^+, etc. fragments like in the earlier photofragmentation study. Fragmentation of C_{60}^{2+} yielded only C_n^{2+}, not C_n^+ in contrast to polycyclic aromatic hydrocarbons (PAHs) which undergo charge separation reactions. In addition to collision-induced dissociation (CID), surface-induced dissociation[24] was also performed. Collisions on both silicon and graphite surfaces showed that C_{60} does not dissociate appreciably at collision energies in the range of 200–300 eV. A number of studies have confirmed this unusual stability and has been attributed to the 'resilience' of the molecule. Similar properties have been predicted for its hydrides also[25]. C_{60} collision also leads to delayed ionization and thermionic emission[26]. Surface collision experiments have been reported on higher fullerenes and metallofullerenes as well[27]. Mass spectrometry has also been used to study thermodynamic properties of fullerenes[28].

In addition to the fragmentation, endohedral complex formation is also observed when high energy collisions are performed[29]. Eight keV collision of helium with C_{60}^+ produced a number of C_{n+4}^+ mass peaks. When the collision gas was changed to ^3He, the peaks shifted by one

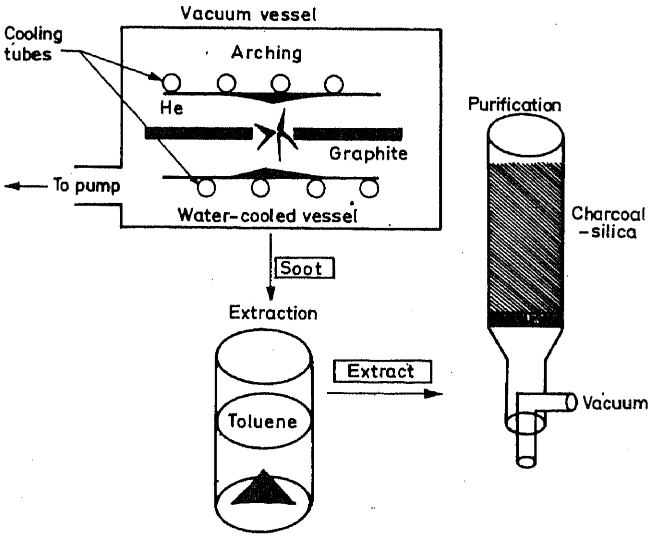

Figure 4. A schematic of the experimental procedure used to make gram quantities of fullerenes. The soot collected after arc evaporation of graphite is extracted with toluene and the soluble material is subjected to chromatographic separation (adapted from Rao, C. N. R. and Seshadri, R., in *Organic Molecular Solids* (ed Jones, W.), CRC Press, in press).

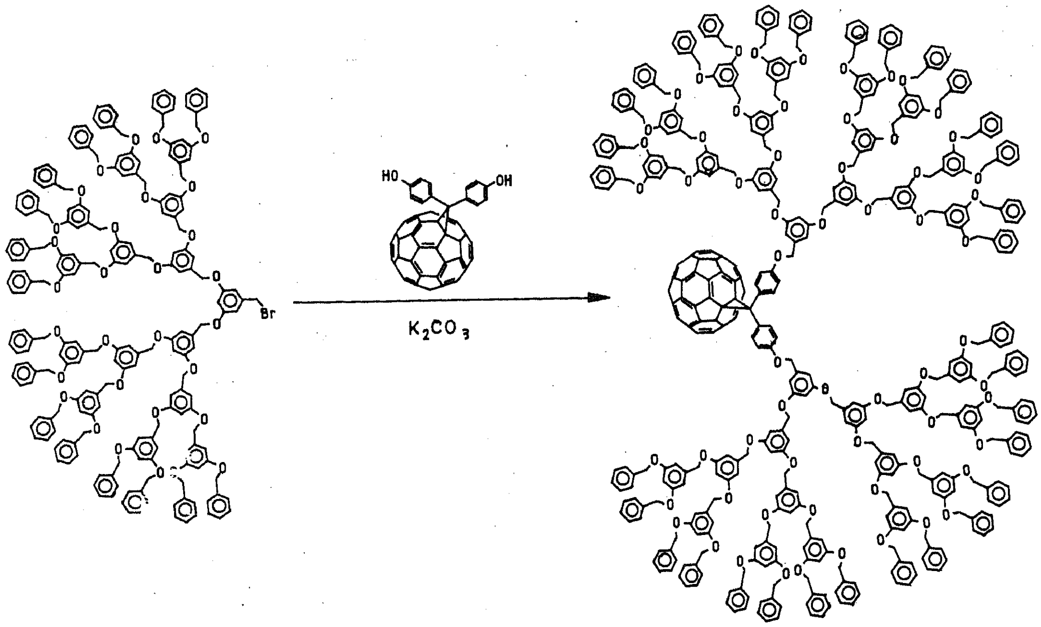

Figure 5. Schematic showing the synthesis of dendritic methanofullerene, using the established dentritic growth procedures (from ref. 132).

mass unit showing that the peak was due to the addition of helium atoms to C_{60}. Others did experiments in different types of hybrid tandem mass spectrometers and confirmed the results. Ion kinetic energy and CID measurements showed unambiguously that $He^+@C_{60}$ (the @ symbolism implies that the species prior to @ is within the cage of the fullerene after the symbol) is an endohedral complex. Similar measurements were repeated with C_{60}^{2+} and C_{60}^{3+}. C_{70}^+ and C_{84}^+ were also shown to form endohedrals. $Ne^+@C_{60}$ and $Ar^+@C_{60}$ are hard to observe in conventional spectrometers due to large energy losses, but they have been seen in specially designed instruments (see also the section on endohedral complexes).

In addition to the work on pure C_{60}, mass spectrometry has been used extensively to characterize the derivatives of C_{60} formed by reactions[30]. The identification of Birch reduction products of C_{60} was done with electron impact (EI) mass spectrometry. The products of C_{70} were also studied by EI. Reaction products with fluorine showed mass peaks at $C_{60}F_{36}^+$ and $C_{70}F_{40}^+$. These products fragment by elimination of F, CF_3 and C_2F_5. Methylated C_{60} showed products with 1 to 24 methyl groups. C_{60} and C_{70} were found to add to aromatic molecules such as benzene, toluene, xylene, anisole and bromobenzene[31]. (See Box 2 for a discussion of the chemistry of fullerenes.)

The fullerene cage itself could include other elements such as boron[32] and nitrogen[33]. Evaporation of boron nitride doped graphite disk produced boron doped fullerenes[32] such as $C_{60-n}B_n^+$ with $n = 0$ to 6. Photodissociation showed that these species are resistant to fragmentation, but reactions with ammonia showed the acidic behaviour of boron atoms. Species such as $C_{60-n}B_n(NH_3)_n^+$ were observed showing that boron has been incorporated into the cage. Substituted fullerenes such as $C_{59}NH$ are now prepared by synthetic chemistry[34].

Photoionization time of flight mass spectrometry has been used to show that clusters of C_{60} are indeed formed during vapourization[35]. Intensity anomalies in the mass spectrum have been detected corresponding to $(C_{60})_n^+$ where $n = 13$, 19, 23, 35, 39, 43, 46 and 55. The numbers suggest that the closed shell clusters $n = 13$ and 55 are probably icosahedra. The magic numbers are similar to the clusters of Xe and Ar. In Figure 6 we show the structure of the icosahedral cluster, $(C_{60})_{13}$.

Fullerenes undergo coalescence reactions in the gas phase. In laser evaporation of fullerene films, it was found that the mass spectra show peaks at very high masses above m/z 720 with enhanced intensities in the range of $(C_{60})_n^+$ (Figure 7)[36]. This proposes the fusion of C_{60} cages to form larger cage structures. The identity of the products was also studied by surface-induced dissociation, which showed that the clusters are hard to dissociate and no parent fullerene is formed. This excludes the interpretation that the peaks could be due to difullerene-like structures. The reaction is supposed to be taking place in the excited dense plasma. Similar coalescence reactions have been reported by others also.

Box 2. Chemistry of fullerenes in the condensed phase

Originally it was thought that C_{60} is an aromatic molecule because there are about 12,500 possible resonance structures. However, it should be remembered that in systems where pentagons are near hexagons, the system avoids double bonds in pentagons. The presence of double bonds in petagons reduces the bond distances, increasing the strain. For C_{60} there is only one structure which avoids double bonds in pentagons. This means that the delocalization of electrons is poor and C_{60} is poorly aromatic[125]. This 'poorly aromatic' classification immediately suggests certain type of chemistry. C_{60} can be visualized in terms of corannulene subunits with two distinct chemical bonds. The 60 6–6 bonds (between hexagons) with a bond length of ca. 1.38 Å have more double bond character than the 60 6–5 bonds (between pentagons and hexagons) of bond length ca. 1.45 Å; which are more like single bonds. This means that the pentagonal rings are extremely strained and insertion of a double bond in the 6–5 ring can bring in instability to the tune of 8.5 kcal/mol (ref. 126). However, describing its chemistry in terms of substructures such as radialene and paracyclene (see Figure 1) does not reflect the chemical reality fully since these substructures are planar while fullerenes are spherical. Fullerenes are strained and continuous and describing their chemistry in terms of a strained weakly aromatic molecule may be more appropriate[127].

Since fullerenes have only carbon atoms, substitution reactions cannot be done with them. However, such reaction could be done on its derivatives. The cages consist of sp^2 hybridized carbon atoms which have –I inductive effect. Therefore, fullerenes are strongly electron attracting and they react readily with nucleophiles and the reactions are similar to those of poorly-conjugated alkenes. The important problem is the number of addition products each reagent is going to bring about. A given product can also have a large number of structural isomers. Added to this, they may have very little solubility in organic solvents. Many of them do not have high stability due to the strain caused by the addition and therefore they may revert to parent fullerene under mass spectrometric examination. Only a few of them crystallize easily so that single crystal examination is possible. Added to this, the study has to be performed with very small quantity of material, often milligrams, although the situation has substantially improved in recent years. Sometimes, the purification procedures leave only tiny quantities of pure compound at the end. In many a case, purification of isomers is very difficult which makes complete understanding of the chemistry extremely time consuming, often impossible. Therefore, a large number of reactions are still not fully studied. However, there are certain investigations which stand out and have resulted in unique products. Because of its comparatively easy availability and due to its unique symmetry, C_{60} has been subjected to more detailed examinations.

Early electrochemical studies suggested that fullerene C_{60} undergoes six reversible reductions[128] corresponding to the complete filling of the t_{1u} LUMO. The early C_{60} chemistry revolved around this high electron affinity of the cage. Adducts of C_{60} with radicals, nucleophiles, carbenes and dienophiles have been reported. Functionalization leading to η^2 complexes of transition metals[129] was the early organometallic chemistry reported. Alkylation of C_{60} leading to methanofullerenes[130] is being intensely pursued by the groups of Wudl, Rubin and Diederich. A detailed account of this can be found in a recent book by Hirsch[5]. These reactions have been shown to result in fullerene polymers[131], fullerene dendrimers[132] (see Figure 5), fullerene-based HIV protease inhibitors[133], fullerene nucleotide conjugates[134] and a number of other potentially useful materials. The electrochemical and photophysical characteristics of fullerene adducts can be technologically interesting. Although the chemistry of C_{60} is intensely investigated, that of higher fullerenes is beginning to attract attention. Because of the presence of large number of non-equivalent bonds, the product distribution is more complex. Nanotubes of carbon are also subjected to solution phase chemistry in a number of laboratories[135].

Another type of reaction which has not been observed before has been the addition of C_{60} and C_2 in the gas phase[37]. In this study, a C_{60} film was bombarded by high energy ions. The mass spectrum of the produced ions shows peaks at C_{60}, C_{60+2}, C_{60+4}, etc. all the way up to C_{108}, the mass limit of the instrument as shown in Figure 7. This suggests that the C_n fragments add back to C_{60} in the region called 'selvedge', the region immediately above the condensed phase where ion/molecule reactions occur. In fact such addition reactions are shown to occur without any activation barrier[38].

Endohedral chemistry of fullerenes

The cavity in the 7 Å diameter buckminsterfullerene molecule has intrigued chemists ever since the discovery

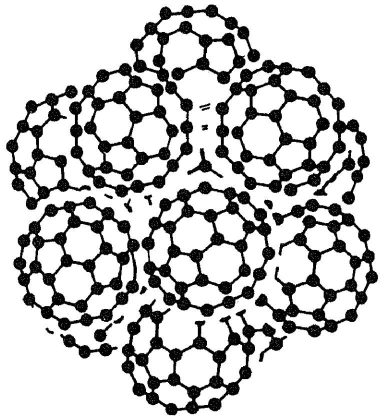

Figure 6. The icosahedral cluster $(C_{60})_{13}$ (from ref. 35).

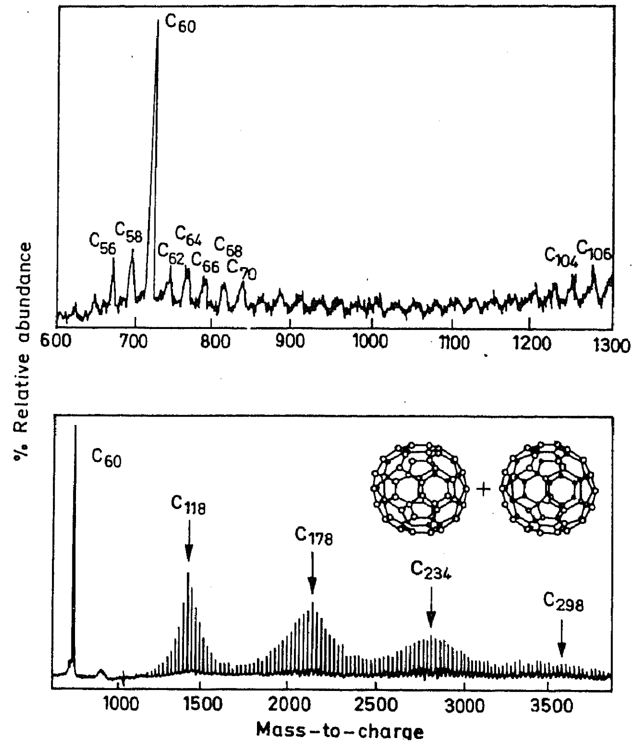

Figure 7. *Bottom*, Mass spectrum of a laser evaporated C_{60} film showing coalescence of fullerenes. Mass peaks are seen at $(C_{60})_n$ (from ref. 36). *Top*, Collision of high energy ions on C_{60} results in the addition of C_2 units to C_{60}. The mass spectrum shown here shows the addition of a number of such species (from ref. 37).

of fullerenes. Later, Smalley and colleagues[39] showed that laser evaporation of graphite impregnated with La_2O_3 can give La@C_{82} and the product could be extracted in toluene. The molecule was shown to be a radical exhibiting EPR hyperfine structure[40]. Mass spectral evidence for other endohedrals was soon available. High-energy collision experiments involving fullerene ions and noble gases were shown to result in noble gas encapsulated fullerenes[29]. For a substantial period of time, characteristic mass spectral fragmentation pattern was the only experimental evidence for the existence of endohedral fullerenes. The fact that He could be put into the cage prompted Saunders to probe the chemistry of endohedrals through ^{3}He NMR[41]. Rg@C_{60} (Rg = rare gas) molecules have been synthesized by a high temperature–high pressure method[42]. Studies showed the high diamagnetic shielding of the inner C_{60} surface indicating the existence of a high degree of aromaticity[43]. The ^{3}He endohedral chemistry is now being extended to the study of derivatives of C_{60} (refs 43, 44) and isomers of higher fullerenes[15]. Results show that there are at least eight C_{78} isomers and nine C_{84} isomers, much higher than originally suggested. La@C_{82}, La@C_{80}, La@C_{76} and a number of Y, Sc and Mn endohedrals have been prepared and purified. The electrochemical, electronic and magnetic properties of these materials have been investigated[45,46]. Gd@C_{82} is paramagnetic down to 3 K (ref. 47). There are theoretical predictions on the various electronic properties of endohedrals but experimental confirmation is awaited. A recent development in the endohedral chemistry is the preparation of Li@C_{60} by low energy ion-beam collision of Li^+ on a C_{60} film[48].

Orientational ordering

Of the several properties of fullerenes, a widely investigated aspect is the orientational ordering. Generally molecules of a high point group symmetry crystallize with a certain degree of orientational disorder. Some lower fullerenes are highly spherical and are held together by weak van der Waals forces, the molecular orientations need not be ordered as in the case of an ordered crystal. This high asymmetry is evidenced by the fact that large thermal parameters are necessary to fit the observed diffraction patterns. This symmetry is the reason why C_{60} had to be fixed with a handle by complexation to refine the atomic positions as was done by Hawkins[121].

In the pure form, because of its high symmetry and weak intermolecular interaction, C_{60} rotates freely in the lattice. This may lead to interesting properties such as the unusual flow of liquids over C_{60} film[49]. This rotation can be frozen at low temperatures, resulting in an orientational ordering. Upon reaction or by co-crystallization, which inhibits free rotation of C_{60} molecules, the atomic positions have been studied. At room temperature, C_{60} gives a sharp NMR signal at 143 ppm and the signal broadens at low temperatures as a result of chemical shift anisotropy[50]. This is due to the dynamic nature of the disorder, the orientational correlation times are of the order of 16 picoseconds. The orientationally

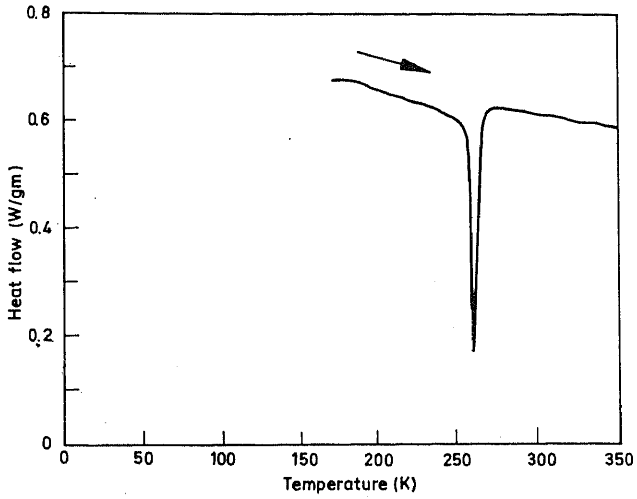

Figure 8. Results of a differential scanning calorimetric measurement of a powder sample of C_{60}. The arrow indicates that the data were taken upon warming (from ref. 51).

disordered fcc phase undergoes a transition to a sc phase at 248 K (ref. 51). In Figure 8, a differential scanning calorimetry trace of the transition is shown. In the sc phase, the rotation exists only along preferred axis. NMR studies have shown that below the ordering temperature the molecules jump between preferred orientations over a barrier of 3000 K (ref. 50). Phase transitions can be modelled which show that simple L-J potentials reproduce experimental behaviour. Charge-transfer effects are, however, important[52]. Neutron diffraction studies indeed provide a strong support to this suggestion in which it is found that the electron-rich bonds of one molecule are located close to the electron poor pentagon rings of the adjacent molecule[53].

Orientational ordering results can be seen in IR and Raman spectroscopies also[54]. A glassy phase has been observed around 80 K when molecular rotations are completely frozen[55]. It may be mentioned that many of the derivatives of C_{60} are also spherical and phase transitions of this type may be observed in them. Recently we have[56] observed such a transition in $C_{60}Br_{24}$.

C_{70} undergoes two phase transitions at 337 and 276 K and these transitions have been studied by IR and Raman spectroscopies[57]. Diffraction studies suggest a monoclinic structure for the lowest temperature phase and rhombohedral or hexagonal structure for the in-between phase[58]. Complete orientational freezing happens only at 130 K. Upon phase transition, the intramolecular phonons undergo hardening, a general feature observed for both C_{60} and C_{70}. Resistivity measurements have proved that there are two phase transitions in C_{60} and three in C_{70} (ref. 59).

Pressure effects

Both C_{60} and C_{70} are soft solids and their compressibilities are comparable to the *c*-axis compressibility of graphite. Upon application of pressure, the orientational ordering temperature increases at a rate of 10 K/kbar (ref. 60). Around 20 GPa, C_{60} undergoes a transition to a lower symmetry structure[61]. Above 22 GPa the material undergoes amorphization and the phase shows evidence for sp^3 hybridized carbon. In C_{70} amorphization happens around 12 GPa and only sp^2 hybridized carbon is seen[62]. This suggests that while polymerization via Diels–Alder addition might be happening in C_{60}, no such reaction occurs in C_{70}. Polymerization upon light irradiation has been observed in C_{60} (ref. 63). Under extremely high pressures, it is possible to convert C_{60} to diamond at low temperatures[64]. Such a conversion is seen in C_{70} also.

At fairly low pressures below the amorphization pressure, the photoluminescence band originally at 1.6 eV undergoes a redshift[65]. At a pressure of 3.2 GPa the band merges with the background, corresponding to the collapse of the photoluminescent gap. This arises due to the broadening of the HOMO and LUMO which is a result of shortening of the inter-molecular distance and concomitant increase in the interball hopping integral. This study has implication to the low temperature electrical properties found in doped fullerenes.

Conductivity and superconductivity in doped fullerenes

Doping with potassium, corresponding to the stoichometry K_3C_{60}, produces a superconductor with a transition temperature of 19.8 K (refs 66, 67) (Figure 9). The K_xC_{60} phase

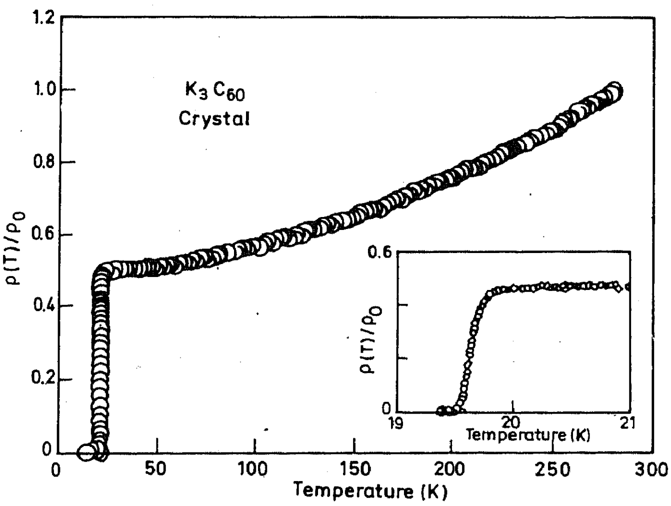

Figure 9. Normalised DC electrical resistivity (ρT) of a K_3C_{60} single crystal. The T_c observed is 19.8 K. ρ_0 is the resistivity at $T = 280$ K (from ref. 67). Inset shows the transition in detail.

was earlier found to be metallic[68]. It is interesting to note that doping graphite with potassium also results in superconductivity, but at extremely low temperatures. However, doping of Na and Li does not result in superconductivity, but other alkali metals do produce superconductors with higher transition temperature (T_c). Mixtures of metals also show the same phenomenon. Because the t_{1u} LUMO is triply degenerate, six electrons can be pumped into C_{60}. Six more can be placed in the t_{1g} LUMO. These electrons can be easily transferred from metal atoms placed in the tetrahedral and octahedral voids of fcc C_{60}. The highest T_c organic superconductor, Rb_2CsC_{60} has a T_c of 31 K (ref. 69). A_6B_{60} (bcc) and A_4C_{60} (bct) phases are non-superconducting[70]. Electron spectroscopy shows that continuous filling of the LUMO happens upon doping but on prolonged exposure, the LUMO band is shifted below E_f – making it an insulator[71]. Upon exposure to transition metals, the *d* states grow near E_f and incomplete metal to C_{60} charge-transfer is observed[72]. Studies show that T_c is a function of interball separation. Thus, intramolecular phonons are responsible for the superconducting ground states in these materials. Superconductivity in A_3C_{60} has been treated as a special case of BCS theory with intermediate electron-phonon coupling[73]. Ca_3C_{60} (ref. 74), Ba_6C_{60} (ref. 75) and Sm_3C_{60} (ref. 76) are superconducting. Superconductivity in higher fullerenes has not been reported, probably due to lower symmetry.

Ferromagnetism in C_{60}.TDAE

Another important property which arises as a result of change-transfer is ferromagnetism. Organic ferromagnetism is a rare phenomenon and is proved conclusively only in a few systems. So far there is only one donor which is shown to produce ferromagnetism as a result of complexation with C_{60}, which is TDAE, tetrakisdimethylaminoethylene[77]. The T_c observed is 16.1 K, which is the highest T_c found for an organic material so far. The material belongs to the monoclinic system with short C_{60}–C_{60} contact along *c* axis[78]. The quasi one-dimensional behaviour has been suggested by susceptibility measurements also[79]. Single electron transfer has been confirmed by EPR[79,80] and Raman studies[81]. Magnetic and electrical transport properties of C_{60}.TDAE have been reported on single crystals also[82]. A number of organic donors have been investigated for potential organic magnetism. Though all of them show pronounced magnetic correlation, no ferromagnetism has been reported[83]. In C_{60}.TDAE, spontaneous magnetization has been verified by zero-field muon spin relaxation technique as well[84].

Optical properties

C_{60} trapped in molecular sieves gives intense red light[85]. Light transmitting properties of C_{60} embedded silica films have been investigated[86]. C_{60} is found to be an interesting material for nonlinear optics, especially for third harmonic generation[87]. C_{60} derivatives would also be interesting in this regard. Third harmonic generation optical susceptibility of C_{70} film is interpreted in terms of the totally symmetric ground state along with two excited states; a one-photon state and a two-photon state[88]. Photoconduction and photoinduced electron transfer, optical switching properties and second harmonic generation are also thoroughly investigated. Several recent conferences have devoted exclusive sessions on the photophysics of fullerenes[89].

Two unusual properties

C_{60} shows anomalous solubility behaviour. Normal solutes show a linear temperature dependence of solubility. In most cases, the solubility increases upon warming. In inorganic systems unusual phenomena are seen due to solute–solvent interactions. Studies of solubility of C_{60} studied in a number of solvents indicate that there is a solubility maximum at near-room temperature[90] (280 K), dissolution is endothermic below room temperature and exothermic above. The results have been interpreted as due to a phase change in solid C_{60} modified by wetting due to solvent. Solubility maximum in organic materials is unprecedented.

Ordinarily materials have a critical point above which the distinction between liquid and solid disappears. The liquid is not seen below the triple point where solid and vapour coexist. C_{60} is one substance in which no liquid phase exists according to theoretical investigations of the phase diagram[91], although the question is still not resolved[92]. If at all such a liquid phase exists, it will be only at high temperatures above 1800 K where the molecules may not be stable.

Carbon nanotubes and onions

Fullerenes need not be found in spherical shape. Upon DC evaporation of carbon, the material deposited on the cathode was found to have tube-like structures of several nanometer length with closed tips[93] such as the one shown in Figure 10. Not only five and six but also seven-membered rings are found in these tubes. There can be single-walled tubes, but most common are multi-walled. These tubes are less susceptible to combustion; therefore, heating in oxygen is a method of cleaning them. The most essential feature of nanotube formation appears to be the presence of high electric fields at the tip of the tubes[94]. The presence of carbon–inert gas plasma make the growing tube end fluid-like. The mechanism is akin to the electrochemical growth processes. The growing tube end closes as a result of instabilities in the plasma. The tubes thus formed have been

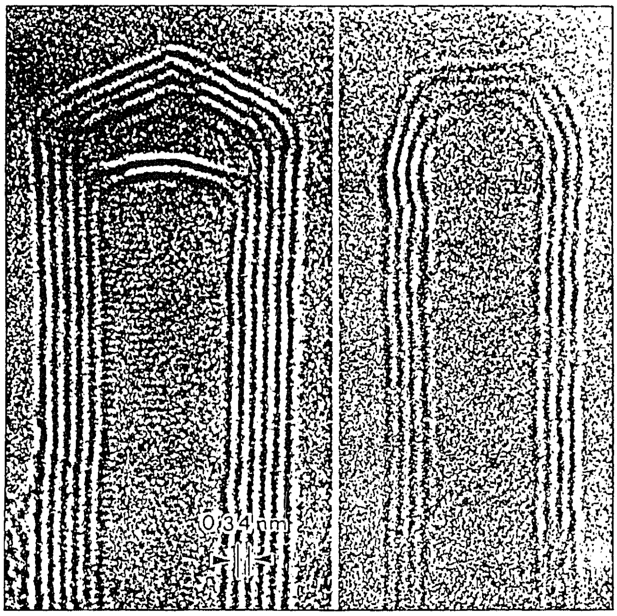

Figure 10. Bucky tubes of different dimensions. The figures are transmission electron micrographs. These are multi-shell tubes. The inner diameter and the length of the tube can vary. Along the body the tube is formed by hexagons and at the ends pentagons are required for closure (from ref. 93).

characterized by X-ray diffraction[95] and Raman[96] spectroscopy.

The structure of nanotubes makes their electronic properties very interesting. Calculations show that these materials could be good electrical conductors[97] and even superconductors (below 1 mK)[98] and the diameter of these tubes can be varied to tune the electrical transport properties. Experimental studies show that the tubes are semiconducting and the behaviour is similar to graphite[99]. Mechanically, the tubes do not have high aspect ratios, making them unsuitable for reinforcing purposes. However, experiments are underway to improve this parameter[100]. A recent application of nanotubes is their use in STM as contact tips.

Tubes can be filled with metals upon opening the tips with oxygen[101]. The probability of making nanoscale wires of metals has enthused many scientists. Lead has been put into the tubes by heating the tubes with lead oxide[95]. Such materials may have interesting magnetic properties[102]. Tube tips can also be opened by chemical methods such as boiling with HNO_3 and if boiled in the presence of metal salts, metals can be incorporated into the tubes.

Carbon onions are also formed along with tubes. These near-spherical large molecules are also found with single or multiple shells and can be filled with metal or metal-carbide particles[103]. Nanoparticles of metals could be placed in these onions so that they are resistant to oxidation, at the same time physical properties such as magentism are unaffected. It has been found that the electron beams of a TEM can be used to open the onion, stuff it with metal and empty it again[104]. It is also possible to use the onion shell as pressure vessels so that the graphite inside can be converted to diamond upon electron beam exposure[105].

Perspectives

The science of fullerenes is still dominated by chemical physics aspects. A quick glance through the literature of the past two years shows that organic chemistry of fullerenes is still in its infancy. The challenges involved in the characterization of derivatives are understandable and complete understanding of a particular reaction requires the application of several tools simultaneously. Therefore, it is very clear that chemical physics and related areas will continue to dominate fullerene research. Of course in the near future, carbon tubules and onions will add more colour to the physics and chemistry of carbon. Materials science of fullerenes is one area which has reached a certain stage of maturity and Indian contributions are particularly noteworthy[106]. Buckyball has brought delight to theorists and the excitements will continue. But it is a pity that in spite of the great many discoveries made, fullerenes have not made long inroads into other areas. C_{60} and its brothers have collectively caught our imagination and in effect it has become the most intensely researched single molecule of 20th century. Probably it may indeed be its greatest legacy.

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Platelet monoamine changes in diabetic patients and streptozotocin-induced diabetic rats

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In the present study we assessed plasma and platelet monoamine content using high performance liquid chromatography (HPLC). The study included 22 subjects consisting of 12 freshly-detected male diabetic patients and 10 age and sex-matched healthy controls. The same parameters were measured in streptozotocin-induced diabetic rat models consisting of controls, diabetic and insulin-treated diabetic rats. The platelet counts were significantly reduced ($P < 0.05$) in rat models as well as human diabetic samples. The plasma norepinephrine (NE) and epinephrine (EPI) concentrations were significantly increased ($P < 0.05$). The platelet showed a significant increase ($P < 0.01$) in NE, EPI and serotonin content. Increase in the plasma and platelet content of neurotransmitters may be due to increased sympathetic function, which is an adaptation for the decreased platelet count observed in our study. The results indicate that changes in the neurotransmitter content of the platelet may be a good index to assess the neurotransmitter status in pathological condition such as diabetes mellitus.

THE role of neurotransmitters in diseased condition has been studied using post-mortem brain samples which are often not reliable since conditions of collection and preservation of such samples are not uniform and there is hardly any choice of age, sex or status of disease. It has therefore become necessary to develop a peripheral model for studying neurotransmitter changes. The blood platelets can be used as a peripheral model since there are structural, morphological and physiological similarities between platelets and synaptosomes[1,2]. Platelets, like synaptosomes are limited by a membrane and contain mitochondria, storage granules, lysosomes, glycogen particles, microtubules and microfilaments[1]. They accumulate monoamines by an active transport mechanism located in the cell membrane and store high concentration of serotonin (5-hydroxytryptamine, 5-HT) in storage granules. The platelets and synaptosomes also possess monamine oxidase which metabolizes serotonin and other amines. Thus we have used platelet as a peripheral model and quantitated NE, EPI and 5-HT by HPLC method to assess monoamine alterations in diabetic patients and streptozotocin-induced diabetes in an animal model.

Three months old male Sprague-Dawley rats of ~180 g body weight were used for all experiments. They were housed in separate cages under 12 h light period and 12 h dark period. They were maintained on standard food pellets and tap water *ad libitum*. Diabetes was induced by a single intrafemoral dose (45 mg/kg body wt.) of STZ prepared in citrate buffer (pH 4.5) (refs 3, 4). The animals were randomly divided into three groups, i.e. controls, diabetic and insulin-treated diabetic groups with 4–6 animals in each group. The insulin-treated diabetic group received a daily dose (1 unit/kg body weight) of Lente insulin. The dose was increased daily according to the blood glucose level. Glucose was measured by GOD-PAP glucose estimation kits (MERCK) (Table 1).

Blood samples from diabetic patients were obtained from freshly-diagnosed cases with a fasting blood sugar level ranging from 150 to 200 mg/dl and with 1.5–2.0 mg urine sugar. None of the patients had any infection or secondary complications and were non-ketotic at the time of sample collection. Only male volunteers between age groups 40 and 60 were selected for the experiment. Patients with any other complications or on drugs were excluded from the study. The controls were age and sex matched.

About 10 ml of blood was collected from human volunteers by ante cubital venipuncture into 1 ml of Alsever's solution (acid/citrate/dextrose pH 5.0). Platelets were prepared according to the method of Steven *et al.*[5] The platelets were counted using a Neubaeur haemocytometer under a phase contrast microscope.

20 µl supernatant from sonicated platelet pellets in 0.1 ml of 0.4 M perchloric acid was injected into an HPLC system integrated with an electrochemical detector to determine the neurotransmitter contents. HPLC separation conditions were followed as previously reported[6]. The monoamine values were expressed per million platelets. 1.0 ml plasma was diluted in equal volume of distilled water. 50 µl of 5 mM sodium

Table 1. Blood glucose levels and body weight of experimental animals

	Blood glucose level (mg/dl)	Body weight (g)
Control *n* = 4	103.28 ± 3.76	174.58 ± 1.69
Diabetic *n* = 6	223.58 ± 20.79*	126.25 ± 4.37*
Diabetic + insulin *n* = 5	140.38 ± 11.24	148.88 ± 3.24

**P* < 0.05 when compared with control.
Values are mean ± S.D. of 4–6 separate animals.

Table 2. Plasma and platelet monoamine content of humans

		Plasma monoamines nmoles/ml		Platelet monoamine content nmoles/million cells		
	Platelet count × 10^9/l	NE × 10^{-2}	EPI × 10^{-2}	NE × 10^{-4}	EPI × 10^{-4}	5-HT × 10^{-4}
Control $n = 10$	327.718 ± 92.15	0.214 ± 0.01	0.039 ± 0.014	0.066 ± 0.02	0.0103 ± 0.003	0.86 ± 0.23
Diabetic $n = 12$	156.745 ± 32.31*	18.96 ± 2.37†	1.78 ± 0.497†	0.63 ± 0.94†	9.2867 ± 1.611†	2.75 ± 0.31†

*$P < 0.05$ compared with controls.

$^{\dagger}P < 0.01$ compared with controls.

Values are mean ± S.D of 10–12 separate determinations.

Table 3. Plasma and platelet monoamine content of experimental animals

		Plasma monoamines nmoles/ml manoamines		Platelet content monoamine nmoles/million cells	
	Platelet count × 10^9/l	NE × 10^{-2}	EPI × 10^{-2}	EPI × 10^{-4}	5-HT × 10^{-4}
Control $n = 4$	245.733 ± 34.0	3.72 ± 0.80	2.39 ± 0.21	17.45 ± 4.53	0.57 ± 1.32
Diabetic $n = 6$	169.800 ± 8.18*	5.08 ± 1.04*	7.29 ± 1.04*	162.08 ± 3.45†	1.579 ± 2.60†
Diabetic+insulin $n = 5$	173.350 ± 9.05*	1.28 ± 0.02*	0.434 ± 0.06†	126.07 ± 15.86†	ND

*$P < 0.05$ compared with controls.

$^{\dagger}P < 0.01$ compared with controls.

ND – not detected.

Values are mean ± S.D of 4–6 separate determinations.

bisulphite was added to it followed by 250 µl of 1 M Tris buffer pH 8.6. Acid alumina (20 mg) was then added, mixed for 20 min, the supernatant aspirated off and alumina was washed with 2.0 ml of sodium bisulphite. To the final pellet of alumina, 0.2 ml of 0.1N perchloric acid was added. The supernatant was filtered and used for HPLC determination of monoamines. 20 µl of extracted sample was injected into a Shimadzu HPLC apparatus connected to a C-18 reverse-phase column. The mobile phase consisted of 75 mM sodium phosphate monobasic, 1 mM sodium octyl sulphonate, 50 µM EDTA and 8% acetonitrile. The pH was adjusted to 3.25 with phosphoric acid and filtered through a Millipore filter. A flow rate of 1 ml/min was used. The monoamines were identified using electrochemical detector. The peaks were identified by relative retention time with standards and the contents were calculated using a Shimadzu integrator (Chromatopac CR6A) interfaced with the detector. Data were statistically analysed by Student's *t* test.

Blood glucose levels of all patients screened were significantly high ($P < 0.05$) compared with controls (diabetic 191.9 ± 7.6 mg/dl, controls 102.2 ± 5.3 mg/dl). Circulating plasma NE and EPI were increased ($P < 0.05$) in human samples. There was a significant increase ($P < 0.01$) in platelet NE, EPI and 5-HT content when compared to controls. The platelet count was significantly reduced ($P < 0.05$) in diabetic patients (Table 2).

Plasma NE and EPI content in experimental animals showed a significant increase ($P < 0.05$), but the increase in plasma NE of diabetic rats was less compared to humans. The EPI and 5-HT content of diabetic rat platelets showed a significant increase ($P < 0.01$). The insulin-treated group showed a decrease in platelet EPI content ($P < 0.01$) compared to diabetic untreated group. There was a significant reduction in platelet counts of diabetic rats ($P < 0.05$) compared to control. The insulin treatment to diabetic rats showed an increasing trend in platelet count but was not significant (Table 3).

The results of the present study showed a diabetic-induced change in the levels of platelet monoamines. In both humans and rats, the plasma NE and EPI levels were significantly high. A similar trend was seen in platelet NE and EPI content of human samples. The content in rat platelets was significantly high, while the NE content could not be detected. The increase in NE and EPI content of human platelets corresponded with the increase in plasma NE and EPI concentration. The same pattern was observed in rat model. But in diabetic rats the increase in plasma NE level was very low compared to human samples. This may be the reason for undetectable level of platelet NE in rats. The increase in plasma NE and EPI observed in experimental diabetes as well as human subjects may be due to the increased output of sympathetic stimulation in both these cases. Previous studies have shown the involvement of sympathetic nervous system in elevating plasma NE and EPI in diabetic condition. The adrenal glands may also be involved in bringing about an increase in EPI[7]. Martin *et al.*[8,9] reported increased serotonin content in human and rat platelet in diabetic condition. Acute myocardial infarction showed a similar platelet 5-HT content[10]. There are also reports of altered NE, EPI and 5-HT in brain regions of diabetic rat models as well as humans[11].

The present study showed a decrease in platelet number in diabetic patients as well as in rat models which supports our earlier report on decreased number of platelets in diabetic rats[12]. The increased content of neurotransmitters observed in plasma and platelets may be similar to the increased sympathetic stimulation observed in our previous studies in pyridoxine-deficient animal models[13]. The increase in monoamine uptake by the platelets can be due to alteration of platelet membrane potential[14]. Kjeldsen *et al.*[15] have reported that a depletion of sodium in the diet can increase the monoamine uptake by the platelets. It appears that the changes in rat and human samples of plasma monoamines are correspondingly reflected in the platelet monoamines. Thus it is suggested that the platelets can be used for measuring the levels of NE, EPI and 5-HT in diabetic condition and the platelet content of these neurotransmitters is altered in untreated diabetes.

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A Mössbauer spectroscopic study of the Pipliya-1 meteorite

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A recently fallen stony type meteorite in Rajasthan namely Pipliya-1 meteorite has been investigated using Mössbauer spectroscopy at 300 K. The obtained Mössbauer parameters resemble pigeonite to a greater extent, among pyroxene. The ratio of Fe^{2+} inner doublet and Fe^{2+} outer doublet confirm the textural interpretations of the geothermal history of the pyroxene. Presence of a weak Fe^{3+} doublet is probably indicating the change in the oxidation state of Fe^{2+} in the meteroite itself because of shock phenomenon.

THE Pipliya-1 meteorite is one of the two Pipliya meteor showers fallen on 20 June 1996 at 20.30 h at village Pipliya Kalan (Lat. 26°2'5": Long 73°56'30"), Pali, Rajasthan, India. A preliminary study of the same has been reported recently[1]. In this paper the Mössbauer spectroscopic study of the Pipliya-1 meteorite is reported.

The Pipliya-1 meteorite has a glossy pitch black crust of nearly 500 μm thickness. Under the fused crust it has typical characters of a stony meteorite. The rock as a whole is a complex breccia consisting chiefly of two types of fragments (type A and type B, welded together), and a few xenoliths[1]. Type A is a fine-grained, gray rock while type B is a coarse-grained, grayish-white rock as reported[1]. The thin sections under petrological microscope reveal[1] that type A is composed of grains of pyroxene (65%), plagioclase (25%), opaque minerals (6%), and some xenoliths (4%). Type B is composed of pyroxenes (55%), plagioclase (40%) and opaque minerals (5%). Pyroxene is clinopyroxene of pale pink colour, nonpleochroic with moderate extinction angles.

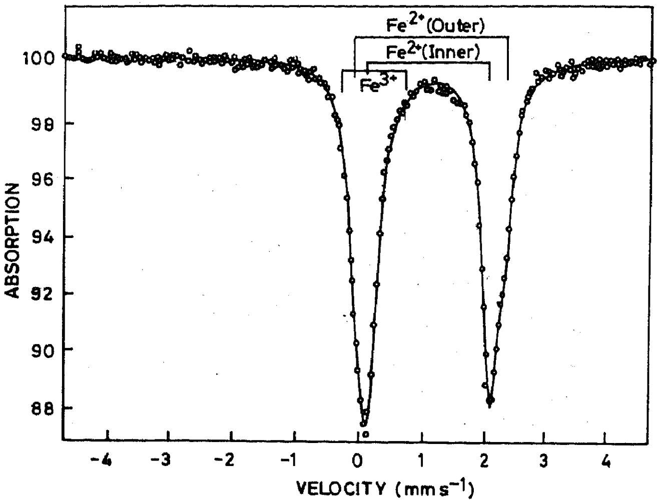

Figure 1. Mössbauer spectrum of rock type A of Pipliya-1 meteorite at 300 K.

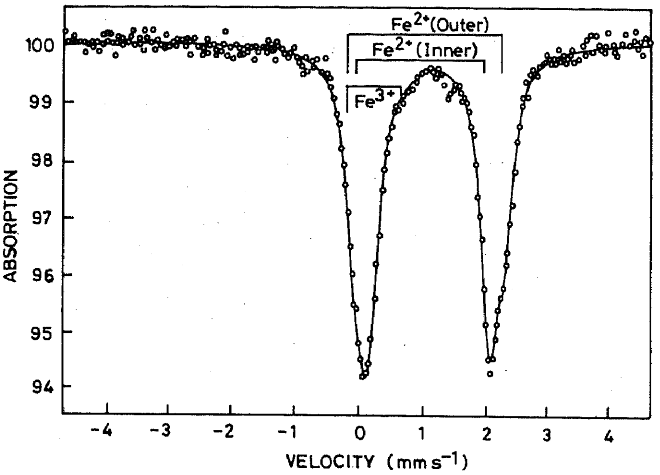

Figure 2. Mössbauer spectrum of rock type B of Pipliya-1 meteorite at 300 K.

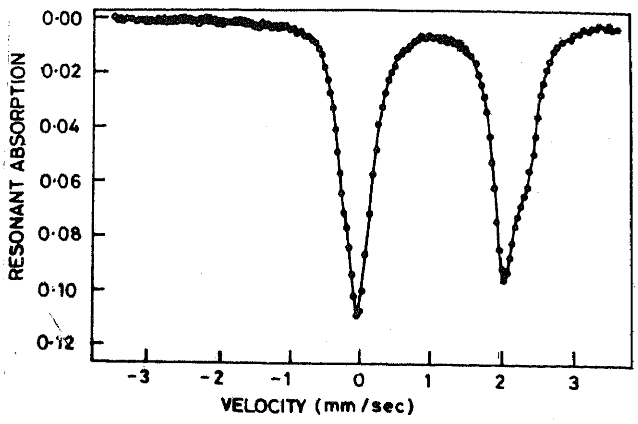

Figure 3. Resonant absorption spectrum of Fe^{57} (295 K) in pigeonite separated from a coarse-grained basalt of the Apollo 11 landing site at Mare Tranquillitatis (ref. 3).

The Mössbauer spectra for both type A and type B have been recorded at 300 K. The thin absorber was prepared by sandwiching fine powder in a sample holder of 2.5 cm diameter. A constant acceleration type Mössbauer spectrometer was used with 25 mCi, Co^{57} in Rh matrix source. All the spectra were computer fitted using a computer program given by Meerwal[2] with some modifications. This program assumes the spectrum to be the sum of Lorentzians. In the present study, the width and the intensity of the two halves of a quaderupole doublet were constrained to be equal. The various permutations and combinations were tried out and iterations were continued till minimum and acceptable χ^2 (goodness of fit parameter) was obtained. The solid lines in the spectra reported here, represent the computer-fitted curves and the dots are the experimental points. This IS (Isomer Shift) values are recorded with respect to the centroid of an α-iron foil. The positions of the individual doublets are marked on the spectrum.

Mössbauer spectrum of type A and type B rocks, constituting breccia of the Pipliya-1 meteorite is shown in Figure 1 and Figure 2. The Mössbauer parameters of the same are given in Table 1. The Mössbauer spectrum of a pigeonite sample obtained from a coarse-grained basalt of Appolo 11 landing site at Lunar is also given in Figure 3 for comparison[3]. The Mössbauer spectra of one terrestrial orthopyroxene (hypersthene) and one clinopyroxene (augite) are given in Figure 4 and Figure 5, respectively for comparison[4].

Several workers have reported the Mössbauer spectra of ortho and clino-pyroxenes[5–15]. The Mössbauer parameters of pyroxenes obtained by the earlier workers are presented in Table 2. In orthopyroxenes, the inner quadrupole doublet (which has smaller QS value) is attributed to Fe^{2+} in M2 site and outer quadrupole doublet is attributed to Fe^{2+} in the M1 site (which has larger QS value). On the other hand, the clinopyroxenes show their inner quadrupole doublet attributed to M1 site and the outer doublet to Fe^{2+} in M2 site. This is because in orthopyroxenes, the M2 site is more distorted than the M1 site, while in the clinopyroxenes M1 site is more distorted than the M2 site.

The Mössbauer spectra of Pipliya-1 meteorite samples show two intense quadrupole doublets but χ^2 values is significantly improved if a very weak doublet is also considered to be present in the fitting. The Mössbauer parameters for both the intense quadrupole doublets are characteristic of Fe^{2+} in high-spin state. On the other hand, a weak doublet indicates the presence of iron in Fe^{3+} state.

Table 1. Mössbauer parameters for Pipliya-1 meteorite at 300 K

Sample (meteorite)	Quadrupole split (QS) (mm s^{-1})	Isomer shift (IS) (mm s^{-1})	Line width (LW) (mm s^{-1})	Relative area (RA)	Doublet	$\frac{Fe^{2+}\ inner}{Fe^{2+}\ outer}$ (Ratio)	χ^2 (per 243 degree of freedom)
Rock type A	1.94	1.09	0.34	71.77	Fe^{2+} doublet (inner)		
	0.93	0.26	0.32	5.25	Fe^{3+} doublet	$\frac{71.77}{21.98} = 3.28$	1.36
	2.40	1.13	0.29	21.98	Fe^{2+} doublet (outer)		
Rock type B	1.92	1.09	0.37	68.01	Fe^{2+} doublet (inner)		
	1.05	0.26	0.27	4.54	Fe^{3+} doublet	$\frac{68.01}{27.45} = 2.47$	1.42
	2.39	1.12	0.32	27.45	Fe^{2+} doublet (outer)		

Table 2. Mössbauer parameters reported for the pyroxenes

Pyroxene	Outer doublet IS (mm s^{-1})	Outer doublet QS (mm s^{-1})	Inner doublet IS (mm s^{-1})	Inner doublet QS (mm s^{-1})	Reference and remarks
Diospside	1.15	2.95	1.15	2.06	Ref. 4 (average of two samples)
Orthopyroxenes	1.16	2.57	1.14	2.04	Ref. 7 (average of seven samples)
Hypersthene	1.15	3.00	1.15	2.14	Ref. 4 (average of two samples)
Pigeonite	1.15	2.5	1.15	2.0	Ref. 3 (calculated from spectra of one Lunar sample)
Augite	1.12	2.72	1.10	2.11	Ref. 4 (one sample)

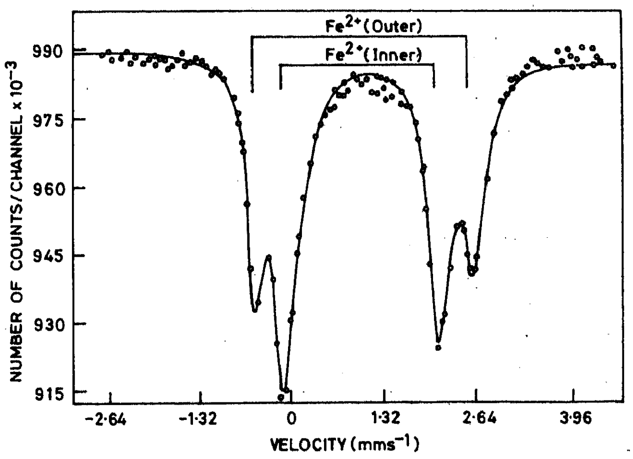

Figure 4. Mössbauer spectrum of terrestrial ordered hypersthene sample at 300 K (ref. 4).

The petrographic studies carried out earlier[1] indicated the presence of appreciable amount of clinopyroxenes in the Pipliya-1 meteorite samples. The Mössbauer parameters obtained in the present investigation (Table 1) also show those values which are characteristic of iron in clinopyroxenes. The large resonance absorption indicates the presence of higher concentration of iron in the sample, which is generally the case in pigeonite because it contains more $FeSiO_3$ component in solid solution. A comparison of the spectra obtained for the Pipliya-1 meteorite with the spectrum of Lunar sample[3] has been made. Both show pigeonite. Although there is no Fe^{3+} in Lunar basalt sample[3], both show similar line shape and Mössbauer parameters, which further strengthen the conclusion regarding the presence of pigeonite. Furthermore, Mössbauer parameters obtained presently (Table 1), do not tally with those reported for orthopyroxenes nor with those of other clinopyroxenes (Table 2).

As far as difference in the Mössbauer absorption in the meteorite samples is concerned, an observation can be made that the relative quantity of pyroxene is more in rock type A than rock type B. This observation is well within the limitation that the thickness of the absorbers for both the types was approximately the same,

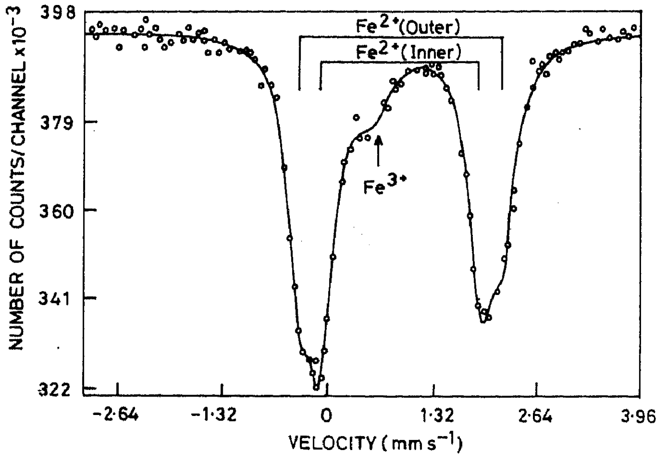

Figure 5. Mössbauer spectrum of terrestrial augite sample at 300 K (ref. 4).

assuming that the 'f-fraction' (recoil free fraction) for pyroxenes in both the types is the same (which is generally the case).

The ratio of Fe^{2+} inner/Fe^{2+} outer is dependent on the geothermal history of the pyroxene[16]. Table 1 shows that the Fe^{2+} inner/Fe^{2+} outer ratio for rock type A is 3.28 and for rock type B is 2.47. It shows that rock type B has more equilibrium distribution and must have undergone slow cooling in comparison with the fast cooling of the rock type A. The conclusion is in accordance with the interpretation in the text.

The fine-grained texture of rock type A is evidence of fast cooling in comparison with the medium-to coarse-grained texture of rock type B showing slow cooling.

A Fe^{3+} doublet of weak intensity has been fitted in the Mössbauer spectra. This peak could have been due to the change in the oxidation state of Fe^{2+} in the meteorite itself because of shock phenomenon. However, this assignment should be considered with caution because the intensity of the doublet is quite weak.

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Altruistic behaviour in *Dictyostelium discoideum* explained on the basis of individual selection

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It is often argued that natural selection acting at the level of the individual may not be sufficient to explain the evolution of altruism. We suggest that before accepting such a point of view in any specific instance, the parsimonious course would be to examine all possible ways in which individual-level selection might act and rule out its sufficiency only when the postulated means for its action are either inherently improbable or experimentally disproven. As an illustration we propose an evolutionary model, based on the individual cell as the unit of selection, for the maintenance of 'altruistic' behaviour by pre-stalk cells in the social amoeba *Dictyostelium discoideum*.

EVOLUTION by natural selection proceeds via the accumulation of successive adaptations that serve to increase the reproductive fitness of an individual measured over its lifetime. Obviously, traits that appear to be detrimental to the fitness of an individual but advantageous to another individual – or to a group – are difficult to reconcile with this view of natural selection. It has been proposed that in order to explain the existence of such traits, one may need to invoke the action of selection at the group, meaning supra-individual, level. Group selection can work in two ways. It may operate directly, between groups[1], or indirectly, within groups – thereby superficially resembling individual selection –, when the groups contain individuals with a significant degree of genetic relatedness. In the latter situation, known as kin selection, kinship makes it possible for the fitness of either of two interacting individuals to be greater than that of the same individual in the absence of interaction[2]. This is because kinship leads to an increase in the probability that both of them carry copies of one or more genes for promoting altruism that are identical by descent. Thus, although an individual may choose to reduce the number of offspring that it has, it may nevertheless benefit genetically if it thereby enhances the reproductive fitness of the second individual to a sufficient extent. Here 'sufficient' means that after devaluing for the coefficient of genetic relatedness, the first individual leaves behind more copies of its genes (albeit indirectly) than it would otherwise. Then, the frequency of an 'altruistic' gene can increase in time and the gene can spread through the population. Indeed, it is often held that such a gene's eye-view is the correct way to think of all evolutionary adaptations, whether in solitary organisms or in social groups[3]. In an extreme situation the first individual may forego reproducing entirely and yet the trait may persist successfully through generations.

Although sound in principle, the concepts of group selection and kin selection have problems in practice[4,5]. Group selection involving non-kin demands special population structures that may not always exist, and explanations based on kin selection can flounder if genetic relatedness is not as high as is demanded by theory[6]. Besides, both models, as well as those based on 'reciprocal altruism'[7], are potentially unstable[8]: they are susceptible to exploitation by 'cheaters', individuals whose contribution to the group is in some sense lower than the benefits they derive from the group. Therefore groups also need to possess the ability to detect cheaters and nullify their effect. The inherent instability of group selection (in which term we also include kin selection) makes it a matter of paramount importance to push arguments based on individual-level selection to their logical conclusion. Except when it can be shown to be inherently improbable or experimentally disproven, such an undertaking has two merits. One, it forces us to think of all possible routes whereby the expected lifetime reproductive success of an individual might be enhanced. And two, it opens up a far richer picture of the evolutionary process for subsequent analysis and testing than a group-selection explanation does; see ref. 9 for an example. We now develop this thesis for the case of division of labour in *D. discoideum*.

But before doing so, it might help to reiterate the essential operational differences between the various models for the evolution of sociality listed above. These differences pertain to the calculation of fitness. For illustrative purposes, let us imagine that we are dealing with a pair of interacting individuals at a time and are comparing expected values of lifetime fitnesses between two situations, one in which cooperation (apparent altruism) is exhibited and the other in which it is not. In a group selection model the average fitness of the pair increases by virtue of altruism. Reciprocal altruism guarantees that each individual gains by cooperation, but only if reciprocity is assured. With kin selection each member of the pair benefits by cooperation because it shares genes with the other (the relevant measure of fitness being inclusive fitness). And under all three schemes a prospective cheater gains most of all unless appropriate counter-measures are implemented. On the other hand, in an individual selection model the only relevant parameter is the intrinsic phenotype of the individual. Given differences in phenotype, reproductive fitnesses will also differ. There is no question of a cheater exploiting the situation because a prospective cheater will also have to take its place, so to speak, in the phenotypic hierarchy – which is equivalent to a fitness hierarchy.

The Dictyostelid slime molds, of which *Dictyostelium discoideum* is the best-studied species, are among the most primitive examples of social organization[10]. Unicellular amoebae aggregate to form a colony within which division of labour takes an extreme form: one subset of amoebae dies and forms a rigid, upright stalk and the other subset forms a ball of spores above the stalk[11]. By making use of a variety of criteria based on staining with vital dyes[11], fluorescence-activated cell sorting[12] and patterns of gene expression[13] it is possible to distinguish between pre-stalk and pre-spore amoebae. Also, the two presumptive cell types are spatially segregated: in the migratory, slug-shaped stage of development that the aggregation goes through, pre-stalk cells occupy approximately the anterior fifth and pre-spore amoebae the posterior four-fifths[14].

After differentiating into spores, pre-spore amoebae come to lie on top of the stalk, meaning at a height above the level of the substratum. It seems reasonable to assume that spore fitness is enhanced thereby, because elevation should improve the chances of successful spore dispersal. On all grounds the pre-spore strategy makes obvious reproductive sense. On the contrary pre-stalk amoebae appear to exhibit altruistic behaviour because in the course of improving the chances of spore dispersal and so increasing the fitness of pre-spore amoebae, their own direct reproductive fitness is reduced to zero. It has been questioned whether such altruistic traits can be accounted for on the basis of individual-level selection (see, for example, ref. 15).

Previous attempts to explain the evolution of sociality in the cellular slime molds have made use, either implicitly or explicitly, of group selection or kin selection arguments[16–19] or have used Evolutionarily Stable Strategy-based reasoning[20] that gives rise to a situation that is also unstable with respect to exploitation by cheaters. There is one experiment that seems to favour kin selection in *D. discoideum*[21], but aspects of its methodology make interpretation somewhat difficult: the authors take the ratio of sorus diameter to stalk height as a measure of the ratio of spore to stalk numbers, and this may not be a good approximation when the stalk is more than one cell thick. In contrast to these earlier models, we now proceed to argue in favour of the proposal that the seemingly suicidal behaviour of pre-stalk amoebae may have a conventional, Darwinian (meaning, individual selection-based) explanation. Under laboratory conditions, *Dictyostelium* aggregations are commonly clonal; but the average degree of relatedness within aggregations in the wild is unknown. Therefore, quite apart from the fact that a high degree of genetic relatedness *per se* would not argue against individual-level selection, the relevance of kin selection in the wild remains a moot question. However, as will be made evident, the reasoning that we use remains valid irrespective of the degree of kinship.

Dictyostelium amoebae emerge from spores, go through a phase of feeding and cell division via mitosis, and proceed to aggregate once the local food supply is exhausted[11]. Our central assumption is that at the time of aggregation there are cell-to-cell variations in phenotypic quality. By quality we mean a parameter that is related to individual fitness; for example, quality may be measurable in terms of the level of metabolizable sugars accumulated by a cell during feeding[22,23]. Quality is, firstly, a relative measure. Secondly, it varies from one cell to another in a quasi-continuous manner. However, for the sake of simplicity we assume that cellular quality can have just two (relative) values. Thus there are high quality (HQ) and low quality (LQ) cells. Our basic contention is that phenotypic selection will ensure that HQ cells stand a high chance of sporulating whereas LQ cells have a low chance of doing likewise. It is evident that such an outcome is intrinsically stable. Genetic differences need not come into the picture at all: obviously, quality may have a genetic component, but as far as the theory goes the cells could be genetically identical. We point out that there is experimental support for a functional non-equivalence between pre-aggregation amoebae as assumed here[12].

A second assumption is that amoebae can assess each other's quality by means of intercellular signals. Signalling may either precede aggregation (for example, via Conditioned Medium Factor, CMF; ref. 24) or follow aggregation (for example, via cyclic AMP; ref. 25 or via Differentiation Inducing Factor, DIF; ref. 26). HQ cells proceed to differentiate along the pre-spore pathway and also attempt to coerce LQ cells to adopting the pre-stalk pathway. The metabolite DIF may be an agent of coercion (see below). The options open to LQ cells are severely restricted. They can choose to stay out of the aggregate and remain solitary, but if they do so they are certain to die[27]. Alternatively, they can join the aggregate and cooperate with pre-spore cells to begin with, all the while exploring opportunities to escape what appears to be their fate and survive, perhaps eventually sporulate. The probability of succeeding in the enterprise is small but not zero: there is evidence that spores can arise from amoebae in which pre-stalk-specific genes were previously expressed[28]. Also, undifferentiated amoebae have been reported in the spore mass and may be a second kind of 'escaper' pre-stalk cells[29,30]. In any event, LQ cells will favour the pre-stalk option even when by doing so their chances of survival are infinitesimal, because the other option – not to aggregate – results in certain death.

DIF (a doubly chlorinated hexanone; ref. 31) and closely related compounds with similar effects are produced by amoebae after aggregation and act as cellular poisons[32]. In low-density assays they cause cells to die and become stalk-like[33]. Curiously, while it is the pre-stalk cells that die and pre-spore cells that give rise to the next generation, the level of DIF is higher in pre-spore cells than in pre-stalk cells[34]. In terms of physiology, the reason behind this is that pre-stalk cells make an enzyme, DIF dechlorinase, that breaks down DIF[26]. But in terms of group (or kin) selection, this does not make sense. Why should pre-stalk cells actively lower the level of a metabolite that is pushing them further, as it were, along the pathway of altruism? On the other hand, individual level selection would suggest that pre-stalk cells (being of relatively lower quality) are inherently more susceptible to DIF than pre-spore cells. Therefore, whereas pre-spore cells are able to continue on the pre-spore pathway in the face of a high local concentration of DIF, pre-stalk cells need to take active steps to break it down in order to prevent early death. Loomis[36] and we[30] have discussed models for pattern formation involving intercellular interaction based on assumptions similar to the ones made here (in particular, Loomis suggests that pre-spore cells may be more resistant to DIF than pre-stalk cells). Recent evidence indicates that there is a heterogeneity even within the pre-spore class; only some pre-spore amoebae exhibit a transient shift to the pre-stalk class (in the sense that their level of intracellular calcium increases) upon stimulation by DIF[35].

A number of testable inferences follow from our model. The existence of a cell-to-cell variation in quality can be tested by sorting presumptive stalk and spore cells at the pre-aggregative stage and probing individuals from each class in respect of fitness-related variables. If the (internal) nutritional reserve available when (external) food supply runs out is a measure of cellular quality, and if DIF plays the role that we suggest it does, one would expect that there is both a higher level of DIF, as well as a greater resistance to DIF, in amoebae grown on a rich medium when compared to amoebae grown on a poor medium. Finally, if DIF acts like an intercellular poison, as it appears to, the operation of individual-level selection would imply that its use as a poison must be a side effect. The primary reason why DIF is made by individual cells would be for their own direct benefit. For, suppose the sole use of DIF was as an agent of coercion against other cells. In that case it would be a metabolic saving, and so of selective advantage, to a pre-spore cell to derive the benefit of the DIF made by other pre-spore cells but to make none itself. Thus a putative 'cheater' prĕ-spore cell that derived the benefits of the DIF made by many other pre-spore cells, but made none itself, would be selectively favoured over the rest. Of what direct benefit might DIF be? One possibility is that DIF protects individual amoeboe or spore cells from microbial attack. It would be worthwhile to look for the presence of DIF in spores. These experiments are currently underway.

In conclusion, we point out that *D. discoideum* by no means exhausts the enormous range of developmental strategies that are seen in the Dictyostelids and their relatives[10,16]. There are species in which a single amoeba can sporulate, species in which the stalk is an extracellular product and species in which cells die and produce a stalk continuously during the course of migration. A careful and detailed study of each case will be needed before we can similarly attempt to explain its evolutionary origins on the basis of individual-level selection.

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Possible evidences of animal life in Neoproterozoic Deoban microfossil assemblage, Garhwal Lesser Himalaya, Uttar Pradesh

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An exceptionally well-preserved microfossil assemblage comprising bacteria, cyanobacteria, algae, fungi, acritarchs and forms exhibiting rather complex/unusual morphology from the black-bedded chert of the Deoban Formation (Garhwal Lesser Himalaya) points to a complex ecosystem of the Neoproterozoic times. The Deoban assemblage is interpreted to have been preserved in the algal-mat facies. Of special interest in this context is the discovery of three problematic forms of questionable affinity. These are comparable to the members of Nematoda and Annelida phyla of the animal kingdom. This suggests that the advent of heterotrophy among animals in its incipient form started in Neoproterozoic which ultimately facilitated the evolution of more complex and advanced communities of multicellular organization in later geological times.

THE late Proterozoic life is represented by Ediacaran soft-bodied animals, acritarchs, prokaryotes, eukaryotes, trace fossils and stromatolites. In recent years many commendable efforts have been made to document the rich fossil records from different parts of the globe which have made the Proterozoic history of life quite impressive and informative. Though the records of plant fossils representing the ancient ecosystem are many[1–6], those dealing with the animal life in the Proterozoic are very few[7–9] and lack the firm basis for assignments of affinities. But during this eon animals must have evolved which ultimately gave rise to well-diversified fauna at the beginning of Cambrian.

The present paper documents three forms from the Deoban Formation of Lesser Himalaya, seen in random thin sections of black-bedded cherts associated with dolostones, which possibly represent the animal organization. Of these, two forms are comparable to some extent with living worms belonging to invertebrate phyla Annelida and Nematoda respectively and the third one is compared with a back-filled burrow. They occur in association with a diversified microfossil assemblage undoubtedly assignable to plant kingdom. The assemblage in general is dominated by benthic population of cyanobacteria, referable to *Siphonophycus*, *Myxococcoides* and *Eoentophysalis*[10–12].

The Deoban Formation is ca. 1000 m thick carbonate sequence ideally exposed near Deoban Hill in the Chakrata area of Dehradun district. The Deoban Formation overlies the Simla Slates possibly with a thrusted contact and is overlain by the Mandhali Formation (Figures 1, 2). The fossiliferous chert samples have been collected from the Deoban Forest Rest House–Chakrata mule track. The lithology is represented by micritic dolostone with intercalations of shale, oolitic dolostone and stromatolite-bearing horizons. These

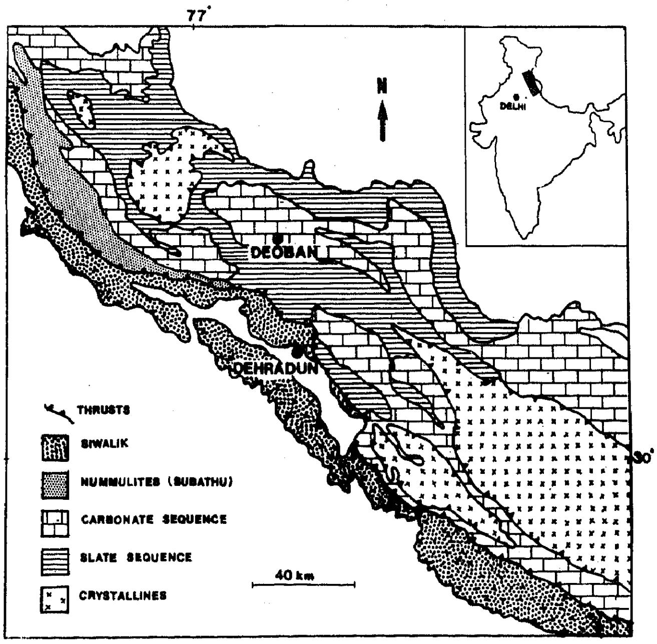

Figure 1. Location and geological map of Garhwal region of Uttar Pradesh (Simplified after ref. 23).

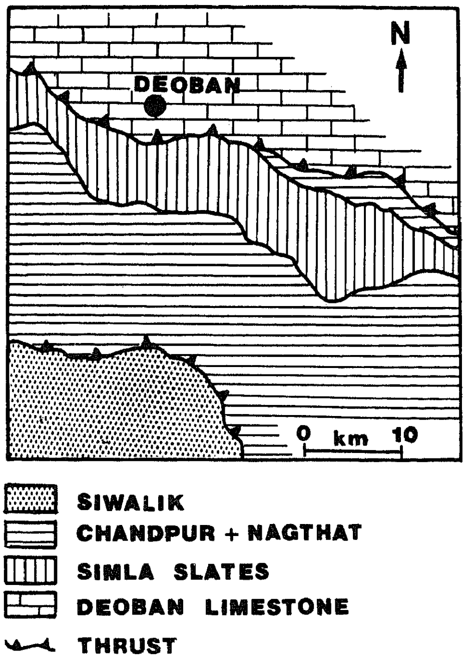

Figure 2. Geological map showing distribution of stratigraphic units in the Chakrata area, Garhwal Himalaya, India (after ref. 15).

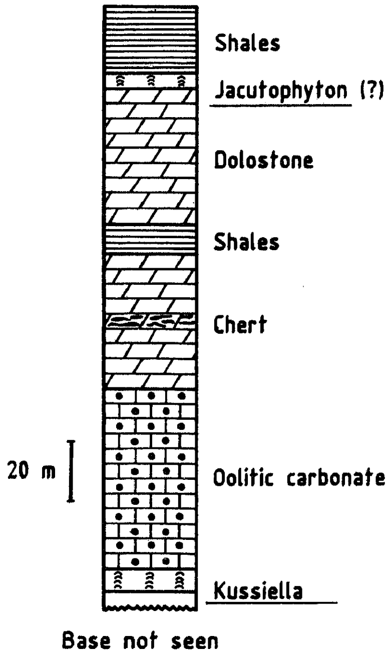

Figure 3. Position of fossiliferous chert in the litholog of the Deoban formation.

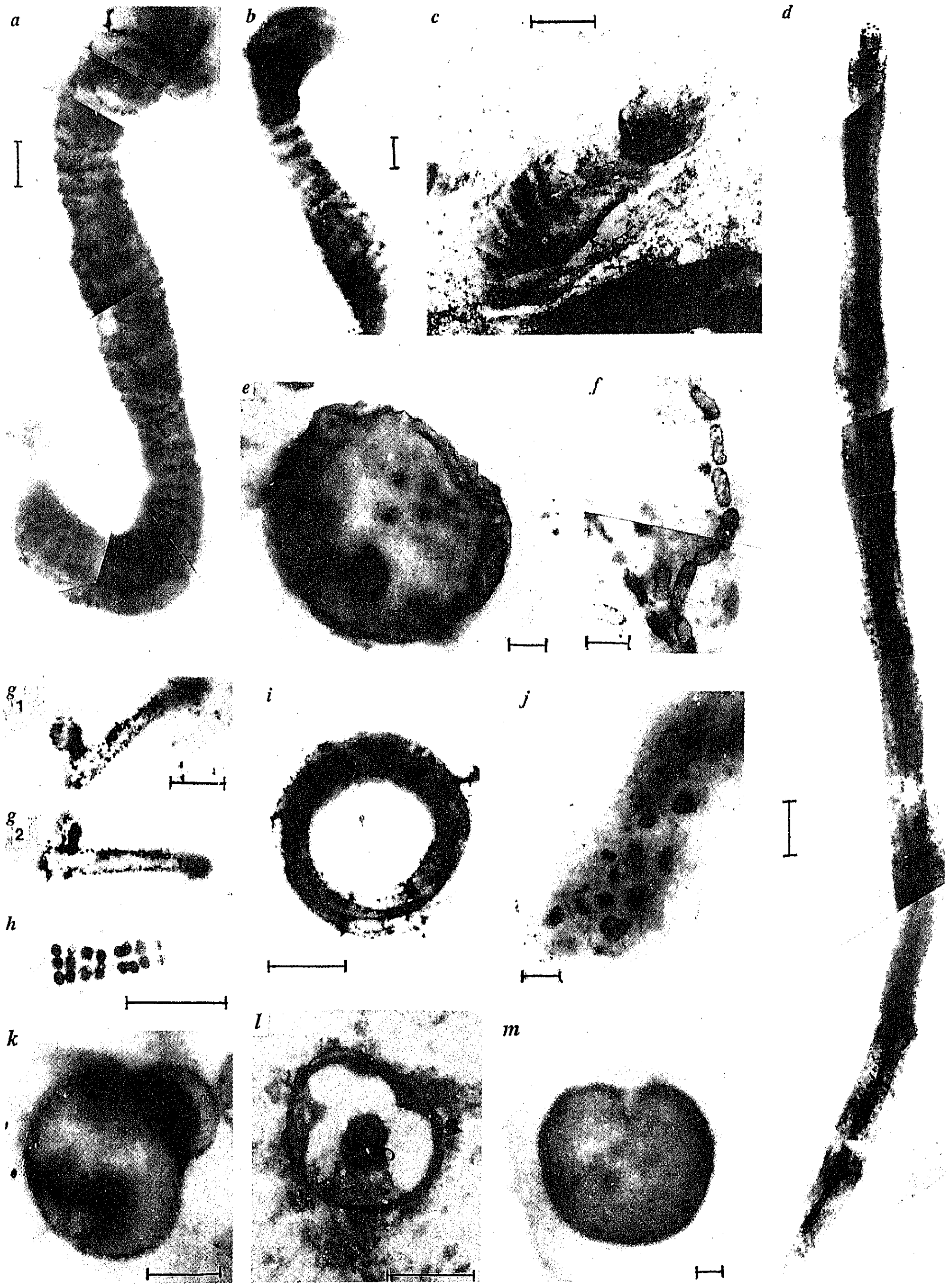

Figure 4. Microfossils from the Deoban Formation, India. All the photomicrographs are from petrographic thin sections of chert housed in Museum of Department of Geology, Lucknow University, Lucknow. Scale bar in all figures represents 10 μm except for the *c* and *l* where it represents 100 μm. ***a–b***, Partitioned worm-like structure, constricted at partitions; ***c***, Back-filled burrow or a microstromatolite-like structure preserved in chert; ***d***, Nonseptate, long, tubular body (comparable to worm of Nematodean affinity) tapered at posterior end. Gut-like tubular structure is seen running throughout its length and projected median part (at the anterior end) and back shifted side margins are also seen; ***e***, Folded walled acritarch; ***f***, Coccus/bacillus bacterial structures arranged in a row; g_1–g_2, Bud-like structure comparable to fungal budding. ***h***, Cyanobacterial or algae-like structures; ***i***, Spirally coiled form (*Obruchevella* sp.) in cross-sectional view where 3–4 whorls are seen. ***j***, Typical arrangement of cells (as in Chlorophyceae); ***k***, Fungal budding (?); ***l***, Sphaeromorph (*Trachysphaeridium* like acritarch); ***m***, Spherical body with 'V' shaped opening.

bands, nodules and lenticles of fossiliferous black chert occur within the carbonates. The position of fossiliferous chert is marked on the lithological column (Figure 3). The chert horizon lies between the two stromatolite-bearing units. The overlying unit contains stromatolite *Jacutophyton* (?) and the underlying unit is characterized by *Kussiella*[13].

The Deoban microfossil assemblage represents a very complex and advanced ecosystem[11,12]. In general the assemblage is dominated by coccoid and filamentous forms of cyanobacterial affinity, though the presence of algal, fungal, bacterial and acritarch forms has also been noticed. Micron-sized coccus and bacillus forms are the smallest forms (1–2 μm) of bacterial affinity (Figure 4*f*). The occurrence of structures comparable to fungal hyphae, bud-like structures (Figure 4 g_1, g_2), typical cellular organization characteristic of chlorophycean forms (Figure 4 *h*, *j*) and large-sized organic walled acritarchs (Figure 4 *e*, *l*) strongly support the presence of eukaryotes among the Deoban microfossil assemblage. In addition, the biostratigraphically significant cyanobacterial form *Obruchevella*[14] (Figure 4 *i*) adds to the complexity of this ecosystem.

In absence of radiometric dates there is no general agreement regarding the age of the Deoban Formation. The age has been suggested from Lower Riphean to Lower Cambrian[13,15]. On the basis of microfossil assemblage especially the presence of *Obruchevella*, large-sized acritarchs and absence of megafossils including trace fossils and noticeable absence of typical Vendian forms (well developed acanthomorphs), the age of the Deoban Formation should be pre-Vandian, i.e. Upper Riphean.

The morphological details of the three problematic forms are: An annulated sinuous and septate filamentous body which is slightly constricted at septa (Figure 4 *a*, *b*). The diameter is almost uniform (10–12 μm) throughout the length except at the ends, which show marked increase in diameter (14–15 μm, 3 specimens traced). The maximum length measured is 280 μm. An elongated tube-like structure running parallel to outer margins is seen in one specimen.

The morphology of these specimens partly resembles Oscillatoriacean filaments (a number of easily identifiable oscillatoriacean specimens present in the Deoban assemblage), but can be differentiated by presence of marked sinuous architecture, broader terminals and annulation. These features make their comparison difficult with any of the extant plant groups and opens an option to compare them with miniatured extant worms belonging to the invertebrate phyla Annelida (Coelomate) or members of the phylum Nematoda known as *Desmoscolex* and *Epsilonema*[8,16,17]. *Rectia costata*[18], a Precambrian fossil from Russia also shows some resemblance with the present form. A megascopic worm-like metazoan (?) fossil named as *Pararenicola huaiyuanensis*[19] from an Upper Sinian Formation of China also shows a comparable morphology (except the dimensions).

A single specimen (Figure 4 *d*) showing a nonseptate tubular, unornamented body tapering at one end (with almost uniform diameter: 10–11 μm) is seen. The other end, probably the anterior one shows a prominently projecting median tube with slightly backshifted side margins. Length measured is 360 μm (single specimen traced).

Eomycetopsis (*siberiensis* sp.)[20], now merged into *Siphonophycus*[2, 21] can be compared with this specimen in diameter and nonseptate tubular body. However, the presence of projected median tube (which continues throughout its length) at one end of the body and tapering at the opposite end differentiates the Deoban specimen from the *Siphonophycus*. These features while supporting its independent entity, indicate some resemblance to *Echinotheristus*; which is placed under the phylum Nematoda[16]. Its projected end can be interpreted as the anterior part comprising mouth and tapered end as the posterior end representing the anal region. The median tube might be its gut as in the case of extant forms.

Living nematodes are usually millimeter sized but can be of microscopic dimensions also. They can be parasitic or nonparasitic in nature. Nonparasitic nematodes are benthic animals and live in interstitial spaces of algal mats, especially the aquatic sediments and soil[16]. This general habitat and mode of occurrence are the additional parameters that support the animal nature of this specimen (Figure 4 *d*) as the Deoban microfossil assemblage is interpreted to have flourished in an algal mat facies[11,12]. The Deoban chert shows presence of well-developed algal laminites comprising abundantly mat building populations; coccoides and filaments.

A single specimen exhibiting semicircular, multilamellate structure where lamellae are curved and each embraces the other. Diameter ranges between 130 and 170 μm, length measured is 380 μm (single specimen traced, Figure 4 *c*).

This particular form shows some resemblance to a cyanobacterial fossil form *Polybessurus*[22] and laminated constrictional features like microstromatolites. The present form can be differentiated from *Polybessurus* by the absence of cone-like structure. To some extent the morphology does favour its assignment to a back-filled burrow[8]: an animal trace-like structure (produced by some worm-like meiofaunal animal that peristaltically moved through sediment) or a microstromatolite.

If these rare and morphologically unusual forms are accepted as animal fossils, they will certainly enhance the significance and complexity of Deoban's ecosystem prevailing at the time of its sedimentation. Despite the controversy about the precise age, an exceptionally well-preserved, highly diversified assemblage of the Deoban microfossils may certainly provide a unique insight into the early stages of animal evolution. It appears that stromatolite-building microbial mat communities played an important role in the evolutionary processes.

The presence of animal life is important as it bears on the viability of the Deoban ecosystem in sustaining the relatively complex producer herbivore (heterotrophs) dominated food chains. This is in contrast to the earlier simple producer food chain based ecosystem that had dominated the global scene for hundreds of millions of years. Once established, the heterotrophy (among animals) may have accelerated the pace of evolution of a still complicated living system that gave rise to complex and more advanced multicellular animal organization in the Late Proterozoic times.

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Ecology and Equity. The Use and Abuse of Nature in Contemporary India. M. Gadgil and R. Guha. Penguin India, 56, Nehru Place, New Delhi 110 019. Price: Rs 135. 213 pp.

At a time when environmental concerns are viewed with distrust and contempt by the policy makers, Madhav Gadgil and Ramachandra Guha's book *Ecology and Equity* comes to us like a whiff of fresh air. There is a lack of proper social scientific analysis on environmental problems in India – more so of any plausible solutions. The authors fill this vacuum by building up an analysis on empirical evidence and when they offer solutions the reader cannot but agree with them.

In this book we come face to face with the real India – the miseries faced by the ecological refugees and the impenetrable iron triangle of beneficiaries, administrators and decision makers.

The real beneficiaries of economic development are only one sixth of the population who are the omnivores. They are rich and powerful. On the other hand, the ecosystem people or the 'biosphere-people' are dependent on nature and hence very close to it. The ecological refugees are large in number and are those people who have been overlooked during the process of large-scale 'development projects'. The authors point out that while there could be no environmental movement which is totally opposed to economic development, for, that would isolate our country on the world map, the appropriate solution would be to integrate and blend conflicting objectives of economic growth and environment protection at every step.

In the first part of the book the authors delve into the past and trace environmental degradation to the colonial days when the British wanted to retain India as a colony purely for economic reasons, namely for its raw materials. As a consequence of breakdown of traditional land and water management practices, there has been a progressive degradation of our coasts, inland water, forests, grazing lands and farmlands. This section is very informative but at times makes repetitive reading. And for scholars familiar with the history of preindependent India, there is nothing new.

The most interesting theme is, however, the classification of the Indian population by the use of resources as governed by the iron-triangle of beneficiaries, administrators and decision makers. State subsidies have given rise to this state of affairs and the omnivores of the iron-triangle are adopting a method of exhaustive resource use at the expense of the ecological refugees and ecosystem people.

That the state machinery has failed abysmally in its performance is evident to everyone, but Gadgil and Guha do not stop there. This seemingly unassuming book is as full of data and information as it is of rhetoric. The second part of the book deals with the 'India that might be' where the authors start with the three main prevalent streams of ideologies namely Gandhism, the Marxist's utopia and the capitalist dream. Gadgil and Guha argue that neither Gandhism nor Marxist philosophy nor capitalism alone can cure the ills of our society. The blend of all the three has been called conservative–democratic–socialism. The emphasis on strong local community from the Gandhian tradition, the democratic institution and private enterprise from liberal capitalism and equity from Marxism have synthesized this conservative–liberal–socialism which is the answer to push India towards better growth and development. With effective decentralization and empowerment of the local people, this could be a solution.

In a country full of contrasts as the authors quote Vikram Seth:

> . . .
> And why is it that Minister's Hill
> And Babu's Barrow drink their fill
> Through every season, dry or wet,
> When all the common people get
> Is water on alternate days?

This book makes a realistic and frank assessment. A truly well written book – a must for every planner and the environment conscious.

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Electrochemistry: The spreading reach of biology

Bioelectrochemistry of Biomacromolecules. G. Lenaz and G. Milazzo, eds. Birkhauser Verlag AG, PO Box 133, CH-4010, Basel, Switzerland. Price: SFR 198.

Biology has intruded into many areas of physics and chemistry and a whole host of subdisciplines now carry the prefix 'bio'. This ubiquitous prefix is found twice in the title of this book which is devoted to the 'bioelectrochemistry' of 'biomacromolecules'. This is the fifth volume in a series entitled 'Bioelectrochemistry: Principles and Practice'. Originally conceived as a comprehensive treatise on bioelectrochemistry by Giulio Milazzo, these volumes have undergone a transformation to a series of multiauthor volumes after his untimely death in 1993.

Electrochemistry has its origins in biology with the work of Galvani and Volta as the forerunner of much of modern day electrochemical research. This volume begins with two comprehensive chapters on the structures of nucleic acids and proteins which form almost half the book (182 pages). Here electrochemistry remains very much in the background. The chapter by Brabec, kleinwächter and Vetterl on nucleic acids is notable for its comprehensive treatment of the electrical properties of these polyanions. The discussion on the behaviour of DNA in electric fields and the dielectric properties of DNA may be useful to readers who normally have recourse only to conventional biochemistry texts, which place overwhelming emphasis on molecular structure and biochemical properties.

The article by Dan Urry on proteins is largely based on the author's own, seminal work on polypeptides, related to the fibrous protein elastin. This chapter makes fascinating reading and much of what is said here may in fact be unfamiliar to those conditioned to discussions of folding based on crystal structures of globular proteins. Although, early structural concepts in this field developed from studies of insoluble, fibrous proteins like collagen, keratin and elastin (and of course synthetic polypeptides), the later flood of information on globular structures has submerged most early work. I found the discussion of hydrophobic interactions and temperature effects illuminating. Urry highlights the marvelous process of 'crystallization upon heating' found in a cyclododecapeptide of elastin. The phenomenon of self assembly into more ordered structures on raising the temperature is indeed well known in the connective tissue proteins. The 'boiled egg' is of course a common everyday example. In more recent times, 'cold denaturation' of proteins has become a fashionable subject for study. This is 'of course the same reversible molecular process' as the heat induced ordering, as pointed out by Urry.

This chapter provides a fascinating extension to mechanochemical effects and electromechanical transduction, with dramatic examples of hydrophobicity-induced pK_a shifts. Which student of biochemistry would not be surprised to hear that it is possible to change the pK_a of an aspartic acid sidechain carboxyl by 6 units (a million fold change in acidity!).

Electrochemistry in its formal form finally appears in two chapters on redox proteins (Hill, Moore and Nabi Rahni) and applications of voltammetric methods (Armstrong). Finally we see cyclic voltammograms, the unmistakable sign that biology is giving way to electrochemistry. This section is instructive for its discussion of methodology and also for the range of applications (Fe–S clusters, proto-linked electron transfer and electron transfer in reductases and dehydrogenases). For the non-specialist reader there is a useful section on the glucose biosensor, now available as a 'glucose pen' for diabetics anxiously following their own sugar levels.

Sulphur is the most oxidizable element commonly found in proteins. Thiol-disulphide chemistry is central to life, as comprehensively reviewed by Gilbert. There is a wealth of information here which is not commonly found in other reviews of the area, particularly redox potentials in diverse proteins. There is yet another chapter on iron-sulphur proteins (Feinberg and Ryan) with a little overlap to earlier sections. The concluding chapter on glycosamino glycans (Pye) appears strangely out of place. While these are undoubtedly changed, the connection to the theme of the book is not readily apparent.

In summary, this is a readable book with many nuggets of useful information. The reference lists are slightly dated and the index is not very helpful. This volume must find a place in libraries that count electrochemists and biochemists amongst their clientele.

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GENERAL

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Review articles (not exceeding 5000 words) are expected to survey and discuss recent developments in a field. They should be well focused and organized, and avoid a general, 'textbook' style.

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1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355–362.
2. Constantine. G., in *Biology of Bats* (ed. Wimsatt. W. A), Academic Press, New York. 1970. vol 1, pp. 319–322.

Acknowledgements should be brief. Footnotes are not allowed except to identify the corresponding author if not the first.

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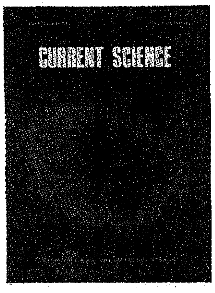

COVER. A developing silkworm embryo (35 hours after egg laying) showing the formation of germ band on the periphery of the egg surface and the vitellophages in the middle region. The specimen is stained with antibodies against PCNA and examined under a confocal microscope. See page 214.

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In this issue

How much water does a tree drink?

The futuristic Hollywood movie 'Water World' depicts a state of world where due to melting of ice caps, our earth is over-inundated with water and the dearest thing on earth for which humankind fights is not gold but soil mined from under water. Fortunately or unfortunately, such a scenario, if it arrives at all, is very far ahead and ahead enough that we need not worry now. On the contrary, among the major resources, what is immediately valued, after the liquid gold, is not soil but water. Our water tables are dipping, water sources are dwindling and despite two thirds of world being covered with water there are scary predictions that water scarcity might catalyse the escalation of inter-human conflicts. (Are we not already witnessing them between our states and with our neighbouring countries?)

Obviously the concern has been about the type of trees we are planting in our living habitats. What if a tree planted in an area consumes more water than the rains received there? We are probably in for a negative balance of the water and we are eating away our future resources. This exactly was the issue that dragged Eucalyptus into the controversy as a 'tree that depletes our water tables'. The sorry state is that we do not yet have an unanimous account of the water budgets for Eucalyptus; for that matter we do not have such accounts for almost all trees around us. And Eucalyptus was probably singularly pointed at.

To avoid these confusions we need to know how much water a tree transpires in a day, in a year and in its lifetime. As usual, simpler the question, the more difficult it is to answer. Anil Graves from Auroville and Pedro Berlinner and Israel Gev from Israel have attempted to analyse exactly this in their paper (**page 196**) in this issue. Following the observations that the water table in and around Auroville is falling down and that spuriously or otherwise this is coinciding with the planting of trees at the Ashram, the investigators were prompted to prepare a balance sheet of water use by the two most dominant trees planted here, viz. *Acacia auriculiformis* and *Peltophorum pterocarpum.*

The most significant contribution of their work, besides computing the water use by the trees, is the use of a non-destructive method of estimating the rate of sap flow through the plant. They did this by giving a heat pulse across the stem at a point and measuring the rate of diffusion of this heat through the sap as it moves through the stem. Since the heat is transported by convection slowly through the sap, the temperature sensing terminal, thermistor, kept at 15 mm above the source of the heat pulse starts registering a gradual rise in temperature with time and once the 'heat wave' passes the thermistor, it starts recording a fall in temperature. The time taken for maximum temperature to be recorded by the thermistor offers an indication of the velocity of sap flow. By suitable calibrations, Anil Grave's group measured the rate of water flow and from thence the total amount of water moved in a given time.

Accordingly they estimated that in a typical day during April, about 110 liters of water flows in *P. pterocarpum* and about 14 liters in *A. auriculiformis*. Extending the computations further and with certain appropriate assumptions, they estimate that the two species show a sap flux of 8150 and 2350 liters per tree respectively in an year. This accounts for about 13% of the rainfall received in the area occupied by the trees which might go up to about 30% if other losses are included. Thus the predominant trees of the Ashram do not appear detrimental for the water table of the area. Independent of the validity of these conclusions their study suggests a way of developing a balance sheet for the water use for trees, an issue that is bothering a lot many crop physiologists.

K. N. Ganeshaiah

Dental composite

The Council of Scientific and Industrial Research of the United Kingdom published in 1980 the results of an extensive survey conducted by it on the worldwide consumption of biomaterials and biomedical devices. Surprisingly, more than 37% of the total expenditure on medical materials and devices was on dental materials. The Sree Chitra Tirunal Institute for Medical Sciences and Technology, Thiruvananthapuram conducted a nation-wide survey of the consumption of biomaterials and devices in India in 1985. Interestingly it was found that 35% of the country's total outlay in the biomedical area went into dental materials and devices, very close to the global average.

The sad fact is that even now most of this demand is met by very expensive imports even for run-of-the-mill materials like stainless steel strips, and zinc phosphate cements.

There is a vague feeling that the Chitra Institute is interested in cardiac and neuro problems only. In fact the Institute has done considerable work in the area of dental care. Products such as dental bands, cements, and fillers have been developed by it over the years.

In this issue, V. Kalliyana Krishnan and V. Yamuna describe (**page 192**) the latest development on light cured radio-opaque composite fillers. UV cured fillers have been around for quite some time. But UV cured systems, especially with the mouth so close to the eyes, pose a potential hazard. Light cured composites are relatively new even abroad.

It is encouraging that this expensive and sophisticated material imported till now can be indigenously produced.

A. V. Ramani

NATIONAL INSTITUTE OF IMMUNOLOGY
NEW DELHI 110 067

WANTED BIOMEDICAL SCIENTISTS

The National Institute of Immunology, New Delhi, would like to recruit **Biomedical Scientists** holding Indian citizenship as investigators leading their own groups responsible for research projects and teaching in the biomedical sciences. A demonstrated record of scientific productivity in the form of peer-reviewed publications in scientific journals of repute and/or internationally valid patents is essential, as is some indication of independence in scientific thinking. At least four years of post doctoral experience would be desirable.

Academic fields

The Institute's primary goal is to gain insights and understanding into the interplay of the immune system and diseases so as to facilitate the development of new approaches to their amelioration. Accordingly, interests and ideas in the study of mammalian systems at the level of transcriptional regulation, signal transduction, cell biology, cellular biochemistry, developmental biology, physiology and structural biology especially in the analysis of the immune system will be considered with much interest. Research areas involving host–pathogen interactions, covering the entire range of expertise and interest from the statistical and epidemiological to the cellular and molecular levels, will also be specially welcome. The Institute also invites scientists in the areas of bioinformatics and of primate biology to apply; scientists in these areas will be asked to function as central 'Service' facility resources in addition to their research as principal investigators.

Remuneration

Appointments offered will range from staff scientist III upwards (equivalent to university positions of lecturer or assistant professor upwards). Length of experience and the quality of the scientific productivity record will be major factors in deciding the level of appointment as well as the salary offered. Shared laboratory space and some initial intramural funding will be provided. Investigators are expected and will be encouraged to bring in extramural project-based funding for research expenses. Some housing is available on campus.

Application

Interested people are asked to send in a detailed biodata providing details of qualification, professional experience /achievements, positions held, with copies of notable papers where appropriate and the names, addresses, fax numbers, telephone numbers and the electronic mail addresses of three potential referees addressed to the Director, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110 067. People working in Govt/Autonomous/Public sector institutions within India are requested to have their applications forwarded through proper channels. The applications from candidates, residing in India, should accompany, except in case of Scheduled Castes/Tribes, a bank draft for Rs 15 in favour of the Director of the Institute. Out station candidates called for the interview shall be paid single second class rail fare to and fro by shortest route on production of the proof of the same. **Those who have already applied in response to the advertisement released in other issues, need not apply again.**

CURRENT SCIENCE

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CORRESPONDENCE

Science education

I have read with interest Modak's article (*Curr. Sci.*, 1996, **71**, 960–967) on a subject which has been uppermost in the minds of many biologists. He should be congratulated on giving shape to the teaching of such an integrated course in life science in our universities. The suggestions made on the structure of the course, in his well thought out table on pp. 965–966, are very comprehensive, making room for choosing specialized streams and optional courses. Perhaps one can add that inclusion of field work in the 4th and 5th years would make the somewhat overweighted theoretical portions in each year a more holistic approach to a 5-year M Sc programme. Creativity can come, in a large measure, by exposing young minds to the marvels of Nature in addition to what they can get within the four walls of the laboratory.

The observations on managements and elected governments made by Modak are essentially what exist today. His remarks on teachers thriving on private coaching classes and tuitions while disregarding their professional obligations in the classroom and laboratory summarize the general apathy to the maintenance of high values in education by the teaching community. Many of us have been silent observers of this new and undesirable trend.

The nationwide debate on the pros and cons of the National Science University (NSU) has done us good in that it has raised hopes that, some day, in the not too distant future, a bold and imaginative government will muster the political will to overcome any obstacle in the establishment of the NSU. I, for one, live in the hope that Modak's proposals need not wait for the day NSU becomes a reality before this century is out. If the Vice-chancellors of all our universities press for implementation of the proposed NSU, it would be an achievement. I cannot understand why, if there can be exclusive universities for most of the Indian languages (and now the one contemplated for Urdu), and also for professional streams like Agriculture, Medicine, Veterinary sciences, what is the problem in an NSU exclusively for basic sciences and mathematics?

I would urge the Vice-chancellors of Indian universities to catalyse the UGC and the Inter-University Board to initiate a plan of action for this 5-year integrated course not only in the life sciences but also in other disciplines of the physical sciences. The unseen hand behind all the rush to seek admission to professional courses is really the parental anxiety to see their wards take to, willy-nilly, medical and engineering courses. If this psychology is to undergo a change, scholarships/fellowships have to be offered liberally to assist the discerning science students to opt for a career in science of a very provocative type (which the integrated Masters' course can offer) albeit parental pressures.

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I was delighted to read the article 'Science Education in Indian Universities: Proposal for a 5-year integrated M Sc course in life sciences', by Sohan P. Modak (*Curr. Sci.*, 1996, **71**, 960–967). His concern and caution coupled with a positive proposal for a potentially beneficial course could be welcome by all concerned but it is only implementation that has always been a 'bottleneck' in this country. I am surprised that the author is unaware of an ongoing near-identical programme in life sciences at Bharathidasan University, Tiruchirapalli existing for nearly a decade now. This 5-year integrated programme, which is the brain-child of its earlier Vice-Chancellor A. Gnanam, attracts over thousand plus 2 completed applicants each year for the entrance test in which only 24 get selected. As the programme is that of the University, funded by the State Government, nearly 70% of the seats are reserved for various communities, leaving only 30% for open competition. If some central agencies come forward to fund this programme, nearly the reverse could be the situation. Although the students out of this programme prove infinitely superior to regular postgraduate students from other colleges and universities and have been successfully placed in academic institutions and industries in India and abroad, the programme cannot be considered a total success because of the usual bottlenecks. Among the important ones are – the lack of proper funding for recurring and non-recurring expenses in running the course, lack of adequate faculty and infrastructure, etc. We often have to be content with excellent proposals and programmes which cannot be implemented in this country with its complexity of problems and highly varied priorities of the powers that be.

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CORRESPONDENCE

And now about urban langurs

Correspondence from Jayashree Datta 'Observations on urban rhesus monkeys' (*Curr. Sci.*, 1996, **71**, 941) makes interesting reading and deserves further study. In the same tone here is an observation about urban langurs which probably draws our attention on another aspect of human activity, viz. cutting trees.

I observed a drastic change in the behaviour of langurs (black-faced monkeys) around my town in Dharwad district of Karnataka. They seemed to have become cleverer (?) and bolder in a short period of about 20 years. Earlier whenever a troop of langurs descended on our kitchen garden, they used to sit on the roof top and wait for an opportunity to safely enter the garden. As soon as someone came close by or climbed the terrace they would run away and find another roof top some 100 metres or more away. They were generally timid, but they might have attacked the same garden the next day.

To my surprise I found that now they are not afraid and even sit on a neighbour's tree only 10 metres away. They skillfully avoid stones thrown at them but sit on the tree very obstinately. This behaviour is seen with several troops and is quite common. How did their behaviour change in such a short period? When I was talking to others who also had observed this change, an explanation was given and appears plausible: The forests in the area have been converted to agricultural land. The cultivation depends on rain and hence the land is barren after harvesting. The irrigated land (mostly well irrigated) is very well protected and monkeys are driven out of such areas. The trees (usually tamarind and some neem) around the town which used to shelter these monkeys have gradually disappeared. Hence out of sheer helplessness, they have learned to sit even at a close distance and avoid stones or any other missiles thrown at them. The vacant area in town also has decreased and the monkeys have to live closer to the humans.

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OPINION

Impact of the new economic policy on science and technology in India

P. N. Andhare

The implications of new economic policy and liberalization for Science and Technology in India are discussed. The result of two conditions imposed by Govt Agencies before sanctioning of financial support is highlighted and a strong plea is made for withdrawal of these two conditions. The perils of evaluating S&T based upon profit generation instead of quest of knowledge are emphasized.

A cornerstone of the Government economic philosophy in the wake of liberalization and new economic policy is its STRONG BELIEF that the 'market' can be allowed to be the master, monitor, judge and guide of all its economic decisions. Having watched the stagnation of the last fifty years, one cannot find fault with this philosophy. Nevertheless, it needs to be pointed out that however wholesome this belief may be in the area of production, services and utilities provided by the state, blind extension of this belief to all segments of society could lead to an unmitigated disaster. This realization finds an echo in the increased allocation for health, education, infrastructure, etc. in the last budget. A struggle is, therefore, on in the minds of policy makers of India to identify sectors, where the influence of the 'market' has to be curtailed, lest it should become detrimental to the national interest.

Resource crunch in S&T

It may sound as incredible if a scientist were to suggest that market forces should not be allowed to dictate the growth and development of science and technology in India. Very few realize that science has its own limitations and that it too needs careful nurturing, it needs inputs of an order that cannot be provided by 'markets'. It is precisely for this reason that all nations stand solidly behind their S&T organizations, protecting them against the vicissitudes of market vagaries. However, under the influence of liberalization and new economic policy, the Government of India has embarked upon a strange scheme – which is designed specifically to foster S&T in India, but which ends up scuttling the very ship of S&T in India.

Critics would hardly agree. DST and DOE will be cited as examples of Government's strong commitment to S&T in India. The public at large is also impressed by financial allocations for these departments – viz. around Rs 500 crores p.a. As often pointed out, the resource crunch in Indian S&T establishment is nothing but a 'wolf's cry', with both DST and DOE returning unused crores of rupees annually earmarked to support S&T activities in India.

Why should Indian scientists complain about a resource crunch, when DST and DOE's annual grant go abegging for want of worthy project proposals – is a legitimate question that needs to be answered. A similar cry was heard last year from private sector about lack of liquidity in markets – though both Government of India and Reserve Bank vociferously denied this. This happened due to Government's decision that banks must increase their CRR, i.e. cash reserve ratio resulting in the so-called liquidity crunch making it difficult for private industry to raise short term loans from the banks, though the banks had ample money to lend. This bears a striking resemblance to the resource crunch faced by S&T in India. In our land of paradoxes, our Government is quite capable of killing a fly sitting on one's nose with a hammer and then feigning innocence and surprise at the sight of a bleeding nose.

Flawed policy of DST and DOE

Precisely the same thing is happening in the decision-making chambers of DST and DOE. Both these departments have imposed two draconian conditions on any S&T project submitted to them for financial support. These two conditions are so heavily skewed against R&D that they nip in bud any S&T proposal even before its evaluation on scientific merit. These two conditions are:

(i) A project proposal will be considered for financial support only, if industry is willing to put its money in the project.

(ii) Industry must also give an advance undertaking that it will productionize the R&D resulting from the project proposal.

Both these are eminently desirable conditions. The only difficulty is that they cannot be met in practice. Why should industry put its money in any external R&D when it can as well use it internally for its own profit making? Why and how should an industry give an undertaking that it would productionize R&D results in advance well before the commencement of a project leave alone its completion? This belies all logic, is against the very spirit of R&D and betrays total ignorance of the dynamics of R&D. Industry can never give such an undertaking nor can a scientist ever demand such an undertaking knowing fully well that the success ratio of any R&D project is well below unity. Both these conditions are impossible to meet in India. DST and DOE, privately concede this and officially insist on its fulfillment. This has resulted in virtual strangulation of Indian S&T in the last few years. These two conditions are responsible for the tragicomic situation – resulting in unused grants with DST and DOE on one hand, and unused labs and unutilized scientists on the other hand, in a large number of CSIR and defence labs. As long as these two conditions remain in force, S&T in India will continue to suffer from the resource crunch, despite plenty of money in the coffers of DST and DOE, just as industry is suffering from a liquidity crunch.

Influence

The problem of S&T in India is, however, graver. Unlike industry, it has no lobby of its own to plead for its cause. Science as a body is much less organized. Industry can mount a campaign through CII, FICCI, CHOGAM, etc. – whether it is a question of a liquidity crunch in the market, high lending rates in banks or imposition of the new income tax, MAT. Science enjoys no such clout in India. Science is often an orphan in India. Consequently its simple plea that it is not desirable to judge a S&T project only on the basis of industrial relevance, evokes no response. Expecting industry to put its money in external R&D and giving an undertaking to productionize such R&D in advance is the surest recipe to abort S&T progress in India. It is, therefore, all the more necessary that planners and policy makers of India listen to the real difficulties of S&T in India. They must not delude themselves into thinking that space and atomic energy is all that Indian Science is about. Indian S&T is much wider and broader than ISRO and DAE.

As a short term measure, therefore, DST and DOE must not insist on these two conditions of support from industry. As a long term measure, S&T must be freed from the clutches of market and motive for profit. A hunger for profit is overtaking thirst for knowledge, corrupting young minds in S&T. Knowledge is to be venerated not because it generates wealth. This is only incidental. The magnetism of an unanswered question holds greater charm in the realm of scientists than all the treasure of the profit seekers. The grandeur of science cannot coexist comfortably with the mundane pursuit of profit. Let us not mix the unmixable. This distorted emphasis on profit and market sustainability are no yardsticks to monitor the growth of science. Science is too valuable an asset to be discarded and disparaged because it is not always profitable. Let the new economic policy and liberalization introduce profit motive where it is due, i.e. in production establishments. Let profit not encroach upon the sacrosanct areas of Science and Technology, where value is held in higher esteem than cost. In our zeal for the lure of the new economic policy and blind faith in the golden mantra of profit, let us not forget that tea is best served hot, sherbet is best served cold – and not vice versa.

Conclusion

While it is acknowledged that all sections of society cannot be developed by dependence on market forces, it is argued in this article that science is one such sector of society. Release of grants by Government agencies only after industry agrees to put its own money in any R&D project and gives an advance undertaking that it will productionize the R&D are two conditions which are throttling Indian R&D. The component of knowledge generation must not be allowed to be eclipsed by the capacity for profit generation. Science is sustained more by the quest for knowledge and less by pursuit of profit. Funding agencies should not overlook this fact.

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Southern India develops a hunch back

A. V. Sankaran

According to plate tectonic concepts, Earth's landmasses form a mosaic of more than a dozen major and several smaller plates (e.g. Indo-Australian Plate, African Plate, Pacific Plate, etc.) all floating over the mantle on a plastic or semi-soft layer of rock. The margins or the boundaries along which two or three major plates meet are usually the sites of tectonic activity, i.e. they are the zones of deformation like faulting; or, regions where ocean floors spread and oceanic ridges rise; or, they may be zones of convergence where one plate subducts and another overrides. While the plate boundaries are thus active, the main oceanic or continental crust, away from the boundaries and capping the plates, are usually passive. However, observations over the last two decades arising out of oceanographic expeditions, appear to belie some of these ideas about plate boundaries being exclusive sites of tectonic activity. Studies[1-3] of the Indian Ocean floor, south of the Indian Peninsula have revealed deformation and faulting in a region far from plate boundary and well within the Indo-Australian Plate. A 900 km long split has been created in this once united plate with the resultant two halves now moving in different directions[4]. Similarly, the Tien-Shan region in central Asia, far away from the collision zone, between India and Eurasia is undergoing deformation, currently[5].

Yet another instance of tectonic activity in a region far from plate boundaries has now been reported by Subrahmanya[6,7]. The area described by him is well within the continental plate making up the crust of southern Indian peninsula close to 13°N. Stretching east–west from Mulki on the west coast to Pulicat Lake, a little to the north of Madras, on the east coast, this Mulki–Pulicat Lake Axis (MPA) (Figure 1), marks a region of uplift due to sea-floor spreading along the Indian Ocean Ridge system. He has cited a number of evidences pointing to this uplift.

Uplift of continental land segments produce many geomorphological changes. The most noticeable ones are their effects on the drainage pattern. Rivers in the area may gradually change their courses or even get dismembered. If turbulent enough, the rivers can cut through the strata keeping pace with the uplift of land and develop, as a result, deep valleys or gorges while retaining their original courses unchanged. Also, changes in sea level take place, leading to emergence of land and accretion to the coastline. Besides, the neighbourhood of uplifted regions may experience seismic shocks of varying intensity.

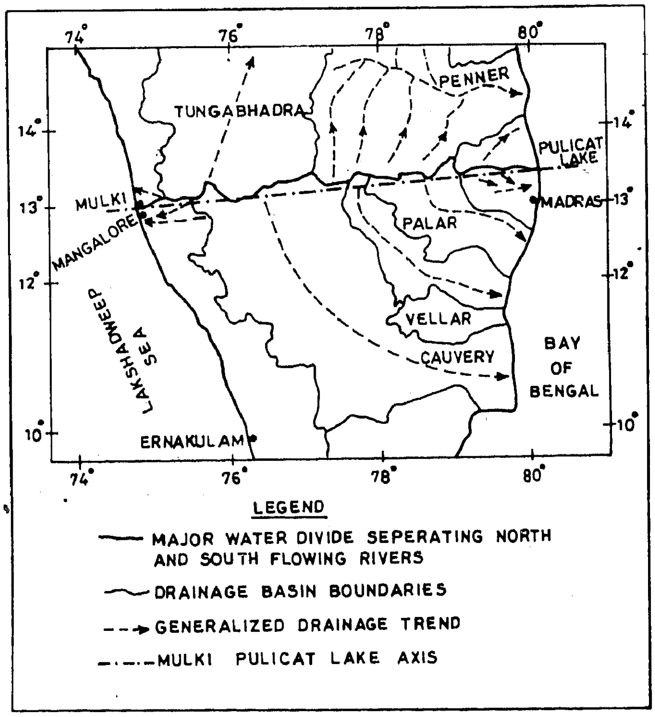

Figure 1. Generalized map of southern India, showing the Mulki–Pulicat Lake Axis (MPA), and the drainage pattern. The MPA is a zone of active uplift and marks a major water divide (after Subrahmanya[6]).

Subrahmanya has recognized all these changes along MPA, commencing from the west coast, passing through the Mysore Plateau to Pulicat Lake on the east. He has observed that the ridge making up the MPA is virtually the water divide and eastward flowing rivers on either side of this line have shifted in conformity with the new slope developed as a result of the uplift, leaving what are termed palaeochannels (ancient channels, now defunct), marking their trends during various stages of the uplift. Noteworthy examples are the easterly flowing Pennar and Palar (Figure 2), which according to palaeochannel evidences, have shifted northeast and southeast respectively as the land between these two rivers gradually lifted up to form the MPA ridge. The author has observed similar river shifts at the western end of the axis also, where the rivers drain into the Arabian Sea. Here, the westerly flowing Swarna, Netravathi and Gurupur, on either side of the MPA, have taken to northwesterly and southwesterly courses depending upon whether they are to the north or south of this axis.

There is also the view that this uplift was responsible for the dismemberment of South India's major river, Cauvery, which once was flowing closer to the MPA, but which subsequent to the uplift, split, leaving the dismembered segments – the Palar and Ponnaiyar rivers, and shifted to the present trend further south, before joining the Bay of Bengal[8,9]. Moreover, there is deepening of the river valleys, and development of river terraces at the western end of the MPA, along the Netravathi River drain-

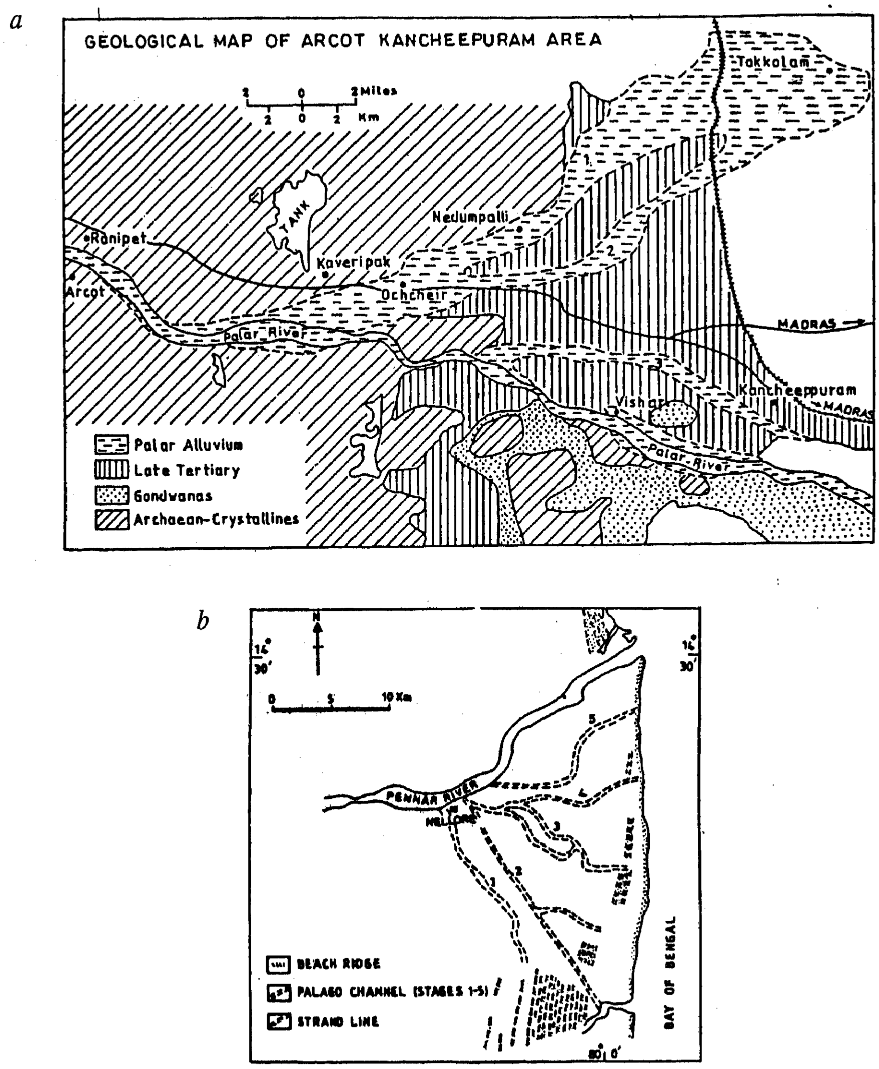

Figure 2. ***a***, Three palaeochannels (1,2, 3) showing the shifts of Palar flowing on the southern side of the MPA; ***b***, five palaeochannels 1–5 of Pennar flowing on the northern side of MPA (after Subrahmanya[6]).

ing into the Arabian sea. Marine shells and sands, pockets of saline waters[10] well inside both west and east coasts, apart from wave-cut platforms at different elevations (seen very well in St. Marys Islands off the west coast), have been noted. These are features arising out of falling sea level. The region close to 13°N has also recorded several seismic events[11] due to 'compression and uplift, related to the north–south oriented regional stress field'; and the Killari and Latur earthquake of 1993, in the adjoining State of Maharashtra, are believed by Subrahmanya to be related to this. Further, he feels that the 'region is likely to experience further upwarp and result in micro-seismicity to meso-seismicity to the south and mega-seismicity to the north of the MPA', thus dispelling the established notions that South India is seismically quiet and stable.

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RESEARCH NEWS

New views on Andes mountains and South American plate movements

A. V. Sankaran

Mountain chains like the Himalayas, according to existing theories, are formed due to plate collisions, like the Indo-Australian continental plate pushing against Asian plate and folding up the intervening area as the lofty Himalayas. However, this theory fails to explain the rise of Andes along the west coast of South America or the Rocky mountains in North America as these continents had no opposing plate to collide against. Two geophysicists, R. M. Russo and P. G. Silver, have recently postulated[1,2] that the geologic forces in the mantle zone below South America is responsible for the rise of the Andes mountains. This view was a sequel to the unusual changes they noticed in the seismic wave propagation, while studying movement of mantle below the western edge of South America. Here the Pacific seafloor, the Nazca plate, subducts eastward (6 cm/year) into the Peru–Chile trench beneath the South American plate, while the latter glides westward (3 cm/ year) over the subducting plate. Contrary to the expected eastward movement of the mantle currents below the Nazca plate, the seismic data revealed movements of these currents, north and south, parallel to the western edge of the South American plate, i.e. at right angles to the expected orientation or flow direction (Figure 1).

The two geophysicists compared this anomalous movement of the mantle currents to that of water swept around a square-fronted boat pushing its way through the sea, implying thereby that the migration of the South American plate westward makes the mantle current flow north-south around the broad front edge of the plate. This motion, as if ploughing through the mantle, generates enormous pressures along the continent's western edge, enough to buckle the region up as Andes mountains. This upheaval is further facilitated by softening of the rocks present along this edge due to volcanic heat from the mantle escaping along the the Nazca plate's sinking edge.

These views received support when similar mantle current movements were observed in their computer experiments simulating a broad-edged continent coursing through mantle. They further found that in both simulated and real continents, the mantle forces exerted maximum impact at the junction where these currents split northward and southward (Figure 1), and as a conse-

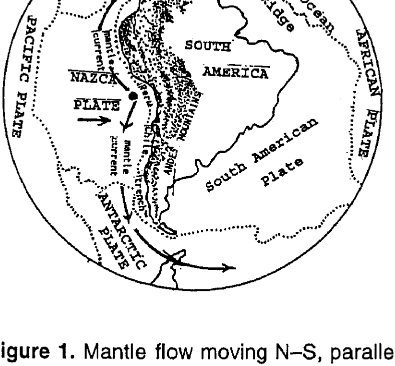

Figure 1. Mantle flow moving N–S, parallel to the edge of westward advancing South American plate. The Nazca plate is subducting into the Chile–Peru trench eastwards.

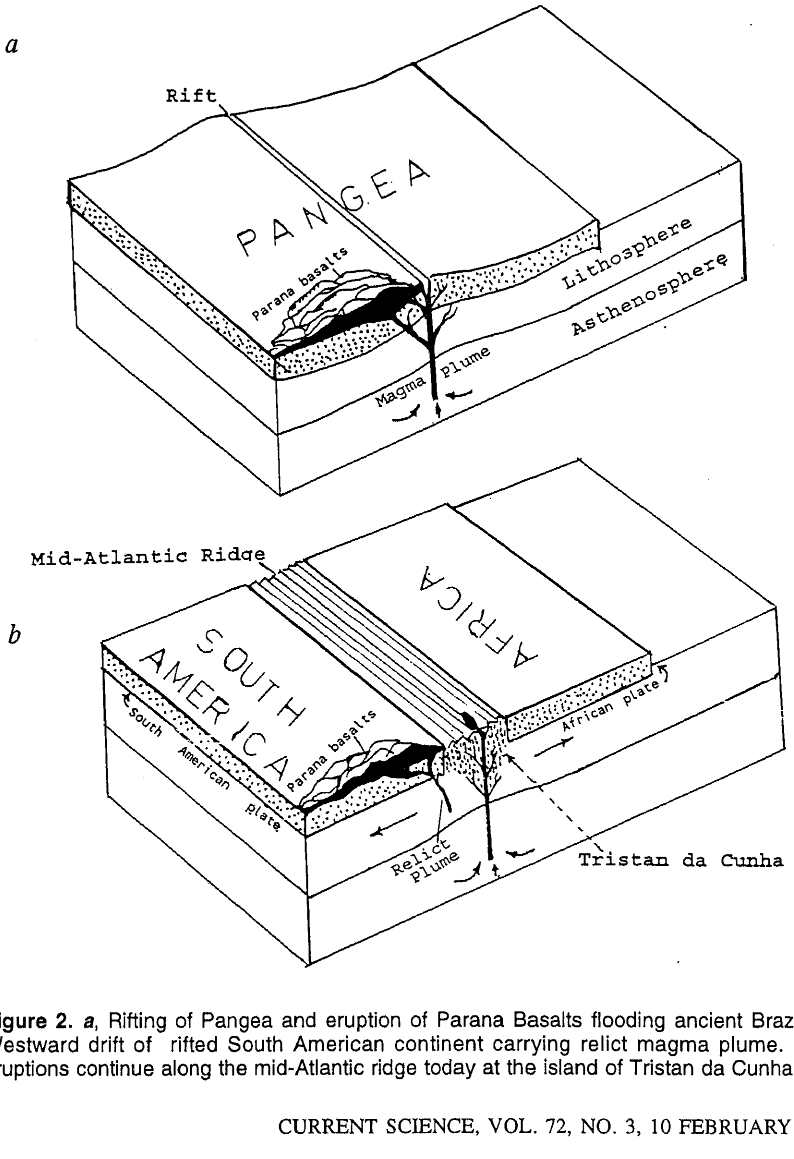

Figure 2. ***a***, Rifting of Pangea and eruption of Parana Basalts flooding ancient Brazil. ***b***, Westward drift of rifted South American continent carrying relict magma plume. Lava eruptions continue along the mid-Atlantic ridge today at the island of Tristan da Cunha.

quence, the Andes mountains are found to attain their widest extent at this mid-point, known as Altiplano. Further, these simulations indicated an eastward curving of the northern and southern tips of the continent, which indeed, they exhibit. The two found parallels in the rise of the North American Rocky mountains, as they too, 100 million years ago, had a similar subduction zone off their west coast, and likewise, exhibit the typical eastward bend, and a wide midsection.

As for the forces driving South American plate, both Russo and Silver are emphatic that this continent is swept westward by deep, wide, mantle currents from beneath the Atlantic Ocean. This view, no doubt, runs counter to the prevailing models of plate movements according to which the low viscosity layer, asthenosphere, separating crustal plates from the mantle, impedes mantle currents to carry the crustal plates along. While the Nazca plate is dragged eastwards by its sinking (subducting) edge, the South American plate is pushed from the rear, westwards, over the Nazca plate, by the emerging new crust – the mid-Atlantic ridge. This conventional 'ridge-push' view is very much doubted by both Russo and Silver who consider these forces alone are too inadequate for the build up of stresses and feel that these are augmented by the underlying mantle currents pulling to the west. But according to Richardson and Coblentz of University of Arizona, Tucson, the magnitude of drag forces from the mantle, invoked by Russo and Silver, are too small to carry the plate westwards[2]. According to their finite analysis calculations of stress measurements within the South American plate and also in the absence of any E–W stress-build expected if drag forces were significant, they feel that the movement of the South American plate should be essentially due to ridge-push force.

However, the theory, that mantle currents were the driving force received support from new data presented at last year's meeting of the American Geophysical Union, by geophysicists John Van Decar and David E. James of Carnegie Institution, Washington (DC). Using seismic tomography, a sort of CAT scan of the mantle below South America, they evaluated the seismometer recordings of earthquakes, worldwide, over a three year period. From these data on travel times of the waves which are influenced by the composition and temperature of the rock or zone through which they travel, the two were able to detect below Brazil presence of a relict or fossil structure of a magma plume which was once the conduit for the vast basalts (Parana basalts) that erupted in that country 130 million years ago (Figure 2 *a*). Obviously, this relict structure had moved 3000 kms westwards along with the South American plate from the original site (Figure 2 *b*). This is a strong indication that 'deep mantle currents carried both the mantle shaft and South American continent' together, though the eruptive source had still remained fixed as is evident from present day volcanic eruptions at Trista da Cunha in the middle of South Atlantic.

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Split comets and crater chains (catenae)

A. V. Sankaran

There is hardly any planet in the Solar System which is not pock-marked by impact craters. Only about three years back, in July 1994, there was a live spectacle of the Shoemaker–Levy 9 comet (S–L9) crashing over Jupiter, an extremely rare celestial event that had mobilized scientists around the world to test some of their theoretical speculations about impacting bodies as well as impacted planets (or satellites). This comet had split into over twenty pieces during its close approach to Jupiter in 1992; these pieces, however, remained in the same orbit, though at some distance apart. Finally, one after another, two years later, they plunged on to this planet's thick gaseous surface, in a rare display of multiple impacts, generating huge plumes or clouds and leaving scars which gradually diffused into the planet's atmosphere.

While these were the outcome of the multiple crash over a gaseous planet like Jupiter, an event of similar nature by fragmented comets or rubble-asteroids over solid planets like Earth or Mars can result in carving of chain of craters (catenae). In fact, such crater chains produced by comets disrupted by tidal forces when they swept past Jupiter[1,2] have been spotted by the spacecraft *Voyager* on two of this planet's moons – Callisto and Ganymede. The craters on Callisto extend over a distance of 360 km with an average diameter of 24 km, while those on Ganymede are lesser in extent and sizes (Figure1). Now, after witnessing the crashes of S–L9 fragments, the origin of these crater chains, long considered enigmatic, no longer remain a mystery.

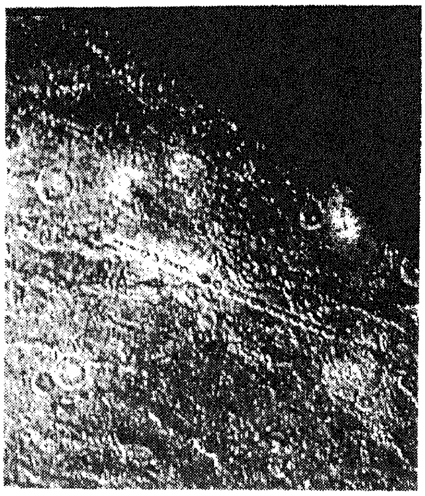

Figure 1. Crater chain on Callisto, the outermost moon of Jupiter, photographed by Voyager Spacecraft. This chain extends for 360 km and contains craters up to 24 km across. The origin of this chain was enigmatic until the impact of S–L9 fragments over Jupiter in 1994.

Recognition of crater chains on Callisto and Ganymede spurred searches for detecting similar ones on Earth and its moon also. Scientists consider their presence on Earth highly likely since our planet is known to have been severely

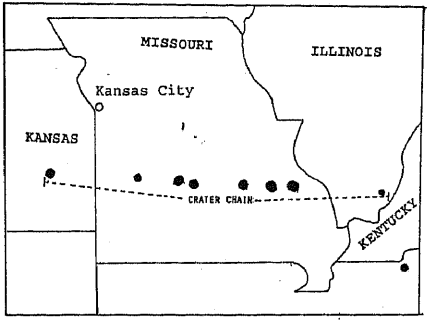

Figure 2. Crater chain extending for 700 km from Kansas to Illinois, USA.

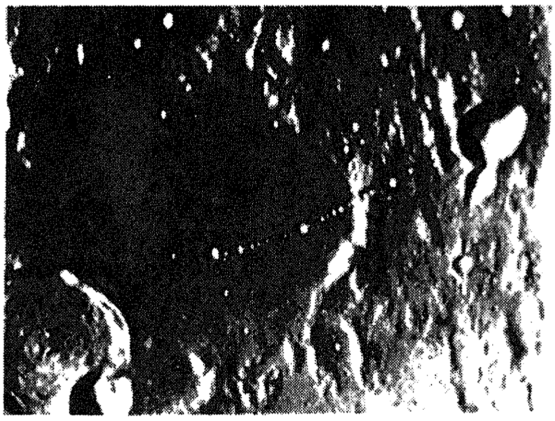

Figure 3. A string of craters in Moon, the Davy crater chain, 47 km long.

battered by meteorites and asteroids in its early past[3], though the likelihood of their unweathered preservation over the intervening millions of years is rather uncertain. In spite of this major constraint, recently it has been possible to decipher crater chains, one in Africa and another in North America through the data gathered by NASA's Imaging Radar Space Shuttle and, also over Earth's moon, from the Apollo Lunar Orbiter photographs[4,5]. The African Aorounga Crater (12.6 km diameter) in northern Chad[3] is now found to have two more craters to its NNE, and probably, a suspected third one also, all hidden below the sands of Sahara desert. They stretch for about 100 km, the largest among them measuring about 17 km across. These craters are believed to have been made by a comet or asteroid which split into fragments up to 1 km diameter, shortly before impacting , some 360 million years ago.

The second discovery[5] in North America is a string of eight craters ranging in size from 3 to 17 km and extending for 700 km from southern Illinois through Missouri to eastern Kansas (Figure 2). Hitherto, they were believed to be volcanic craters, but in the absence of any volcanism in the area and presence of shock produced minerals[6] at the site, they are now considered as coeval impact craters, about 330–310 million years old and created by a 'string of asteroids or cometary objects produced by breakup within the inner solar system[5]'. One more proven impact crater about 6 km in diameter (estimated age <300 million years) lying further east, some 420 km away, in eastern Kentuky[6], is also suspected to belong to the same Kansas–Illinois chain. Though the African craters too are dated around the same time, evaluation of various other data indicates that the craters in these continents were made by two different comets or asteroids. The crater chains found on our moon (Figure 3) range in length from 50 km (Davy chain) to 250 km (near Abulfeda crater) and are supposed to have been generated by comets disrupted tidally when they passed close to Earth or by rubble-pile asteroids[7].

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Further comments on 'Fertile plants regenerated from mesophyll protoplasts of cold-tolerant rice' (J. N. Gupta, *Curr. Sci.*, 1995, 68, 755–758)

An unfortunate controversy has arisen regarding the above paper published in Current Science in September 1995. This was subsequently the subject of discussion in these columns in February 1996. The matter is closed with the publication of the following correspondence.

– Editors

This is a rebuttal of false allegations levelled by J. N. Gupta in his reply published in your esteemed journal (1996, **70**, 264–265). J. N. Gupta asserted in his reply that the work in question (published by J. N. Gupta and S. N. Gupta of Gorakhpur University in *Current Science*, 1995, **68**, 755–758) was done by him at ICAR Research Complex, Barapani (in my laboratory), it is a part of his Ph D thesis and that I misused my position to publish the same.

The allegations are false and without any element of truth. In fact, J. N. Gupta's claim is self-contradictory. On one hand, he says that his protocol published in *Current Science* in 1995 is entirely different from mine which was published in 1993 and on the other hand, he alleges that I misused my position and published his protocol. In order to resolve the controversy, I have now obtained a photocopy of his Ph D thesis and there is no mention of this work in his thesis. This shows that he had not done this work till the submission of his thesis and consequently; his claim to have done this work in my laboratory is wrong.

H. S. GUPTA

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Barapani 793 103, India

I have the following comments regarding the paper in *Current Science.*

1. The published work in *Current Science* (1995, **68**, 755–758) is not a part of Mr J. N. Gupta's Ph D thesis.
2. I never consented to be a co-author in any of his research papers.

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J. N. Gupta maintains:

1. The work reported in *Current Science* (1985, **68**, 755–758) on mesophyll protoplast to plant system in rice has been carried out by me. I never claimed that the reported genotype is a part of Ph D thesis.
2. I published this work from Gorakhpur University, Ludhiana and Gorakhpur; Punjab Agricultural University, Ludhiana and Directorate of Rice Research, Hyderabad respectively.

J. N. GUPTA

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Comments on 'Interstratified low-Ti and high-Ti volcanics in arc-related Khairagarh Group of central India'

(Asthana *et al., Curr. Sci.,* 1996, **71**, 304–306)

1. In Figure 2, Asthana *et al.* have indicated CaO/$\mathrm{TiO_2}$ ratio for low-Ti basalt and high-Ti basalt, which is not consistent with the REE patterns for both the basalt. In low-Ti basalt CaO content is more than that of high-Ti basalt but the shown negative Eu anomaly for the low-Ti basalt is contradictory in respect to CaO content.

2. Derivation of arc-related magma is possible by thermal plumes mechanism involving a thin crust underplating and also by crust mantle recycling.

3. The authors have shown that two basalts have different REE patterns and have explained that which cannot be due to fractional crystallization/crustal contamination, however, the observed REE patterns can be derived from the crustal contamination and/or by combination of fractional crystallization and assimilation (FCA). From the given REE pattern it is hard to assess the heterogeneity of mantle source. Although the observed LREE patterns can be better explained by different degrees of partial melting of a rather homogeneous mantle source. The authors have put a question mark for LILE which is very important to decide low/high degree of partial melting as well as fractional crystallization. Hence, the degree of partial melting appears ambiguous. If the source is LILE enriched then mantle may reflect crustal recycling.

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D. Asthana et al. reply:

1. CaO/$\mathrm{TiO_2}$ vs $\mathrm{TiO_2}$ and $\mathrm{Al_2O_3/TiO_2}$ vs $\mathrm{TiO_2}$ plots (Figure 2) clearly indicate a

fertile source for the high-Ti basalt (CaO/TiO_2 : 10–4; Al_2O_3/TiO_2 : 18–8)[1]. Assuming a similar source, our dataset[2] indicates that the low-Ti basalt (CaO/TiO_2 : 18–8; Al_2O_3/TiO_2 : 26–18) has formed by relatively higher degrees of partial melting than the high-Ti basalt. However, other datasets[3–5] clearly indicate that the source of the low-Ti basalt was relatively depleted (Cao/TiO_2 : 35–10; Al_2O_3/TiO_2 : 45–15). The low-Ti basalt was probably formed by remelting of a source which had undergone a previous episode of magma extraction[1]. Therefore, Figure 2 of our paper clearly indicates heterogeneity in the mantle source of the basalts which is also evident from their REE patterns (Figure 3). The two cross-cutting REE patterns cannot be obtained by different degrees of partial melting of a homogeneous source[6]. The HREE and Ti depletion in the low-Ti basalt can be due to the following reasons:

i) The low-Ti basalt was formed by relatively higher degrees of partial melting. ii) Mantle wedge of the low-Ti basalt has undergone previous episodes of magma extraction. iii) Mantle residue of the low-Ti basalt has higher proportions of garnet[7] and/or rutile[8].

The LREE enrichment in the low-Ti basalt clearly indicates higher subduction fluxes which is also evident from higher LILE contents in this basalt[9–11]. Pandey has stated that CaO content in the low-Ti basalt is more than high-Ti basalt, but from Table 1 it is evident that CaO content in the low-Ti basalt (7.08 wt%) is less than that in high-Ti basalt (8.50 wt%). Lower concentration of CaO in the low-Ti basalt probably indicates plagioclase fractionation which is also evident from negative Eu-anomaly shown by this basalt.

2. Derivation of arc-related magmas is not possible by thermal plumes or crustal underplating. Arc-related magmas are generated by decompression melting in the mantle wedge, augmented by aqueous fluids derived from the subducting oceanic crust[7,9,12–14].

3. Both the basaltic suites have high MgO-content (12–7 wt%) which rules out the possibility of their crustal contamination[15–17]. MgO > 6 wt% is generally considered as an adequate filter for AFC processes[15]. Therefore, we have not correlated the two suites by AFC. We have put a question mark for LILE because these are potentially mobile elements in altered volcanics such as Khairagarh basalts[18]. Therefore, LILE contents assume no importance in deciphering degrees of partial melting in these basalts. On the other hand, in altered and low grade volcanics, relatively immobile elements such as HFSE and REE assume vital importance in understanding degrees of partial melting and fractional crystallization[18]. LILE enrichment in the primitive arc-related magmas clearly indicates enrichment of the mantle wedge by subducting oceanic crust-derived fluids. Therefore, convergent margins are the zones of crust–mantle recycling.

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Predicting monsoon rainfall and pressure indices from sea surface temperature

Several prediction techniques have been developed[1] to forecast the all India summer monsoon rainfall during the period, June–September. Among them, DWPJ-A, BMBPA-J and A-MR500 are found to be the most useful predictors. Nicholls[2] reported that the SST in the north Australia–Indonesian region (5–15°S, 120–160°E) is useful to predict monsoon rainfall.

Several studies[3–6] reported the relationship between SST in Indian ocean and monsoon rainfall over the Indian subcontinent. But, there is a divergent opinion on this aspect which is partly attributed to the poor quality of SST data in the Indian ocean[7]. Using the latest and high quality data set[8] on SST anomalies (MOHSST.6), an attempt is made here to re-examine the role of Indian Ocean SST in monsoon rainfall.

Data on SST anomalies (SSTA) in the grid, 0–5°N, 80–85°E, for the period, 1967–87 and three different rainfall series (R_1, All India monsoon rainfall (1847–1990) in mm based on arithmetic average of 16 representative stations[9]; R_2, arithmetic average of monsoon rainfall of 32 representative stations[10]; and R_3, rainfall anomalies[11] in cm) have been used in the computations. Correlation coefficients between SSTA and (i) DWPJ-A, (ii) BMBPA-J and (iii) A-MR500, are also computed. Data on DWPJ-A, BMBPA-J and A-MR500 are taken from the earlier studies[9,12,13].

Correlations have been computed with (– 1) year and (0) year lags following the method given by Nicholls[2] (correlations of 0.45 are significant at 95% level). The results are shown in Figure 1.

All India summer monsoon rainfall (R_1, R_2 and R_3) is strongly and positively correlated ($r = 0.75$) with the SSTA in the grid, 0–5°N; 80–85°E during October and November months of the previous year. SSTA in February (0 year) is also well correlated with R_1 (Figure 1 *a*). Negative and strong correlation is noticed between R_1, R_3 and SSTA in November, after the withdrawal of monsoon. This could be due to the advection of heat from west to east through equatorial jet which appears in October–November. The jet deepens the mixed layer depth towards east. This may distribute the heat to deeper depth and results in changes in SST anomalies[14].

SSTA in November (– 1 year) is strongly and negatively correlated (– 0.74) with DWPJ-A (Figure 1 *d*) while BMBPA-J is significantly and positively correlated (0.75) with the SSTA during November and December (Figure 1 *e*). A-MR500 is positively and significantly correlated (0.63) with the SSTA in October (– 1 year) (Figure 1 *f*).

To identify the changes in the correlations with time, sliding window correlations have been computed with a width of 26 years, from 1950 to 1991. Table 1 shows the sliding window correlations between SST and (a) monsoon rainfall (R_2), and (b) pressure indices (DWPJ-A, BMBPA-J). Correlations between April SST in the north Australia–Indonesian region (5–15°S; 120–160°E) and monsoon rainfall (R_2) are also shown in column (d) in Table 1. Correlation between October SST in the grid, 0–5°N; 80–85°E, and monsoon rainfall is significant from 1960 onwards. A similar feature is noticed between November SST (– 1 year) and DWPJ-A. But, BMBPA-J is significantly and positively correlated from 1953 onwards. All the correlations (columns a, b, c) show an increasing trend since 1950 onwards. A similar feature was also reported[1] earlier. The correlation between April SST in the north Australia–Indonesian region and monsoon rainfall (R_2) is significant and consistent throughout the study period and a decreasing trend is noticed in the recent period, 1966–91. It is interesting to see that the correlation during the above period is increased in the case of Indian ocean SST and R_2. This may be due to the warming of eastern equatorial Indian Ocean during the recent decades or due to the strong coupling between ocean–land–atmosphere system during the recent period compared with the earlier period as conjectured by Krishnakumar *et al.*[1]. The correlation between Indian monsoon rainfall and April SST in the north Australia–Indonesian region is found to be 0.55 (ref. 2) for the period, 1950–91. The correlation coefficient between October SST of the previous year in the grid, 0–5°N; 80–85°E and monsoon rainfall for the entire period is 0.40 which is statistically significant at 95% level.

From the overall results, it can be concluded that SSTA during October and November months in the EEIO is useful to predict monsoon rainfall and pressure indices. I am not aware of any earlier study which has shown the relationship between SSTA and pressure indices. It is inferred that the eastward propagating low frequency convective systems (LFCs) which originate in the warm pool region in the equatorial Indian ocean[15] could be the main mechanism behind the relationship of SSTA with different parameters discussed above. Secondly, the SST anomalies in this region can affect the 'walker circulation'[11], which is reflected in the strong correlations noticed between SST and (i) DWPJ-A, and (ii) BMBPA-J. Since the 500 mb ridge location along 75°E before monsoon is controlled by

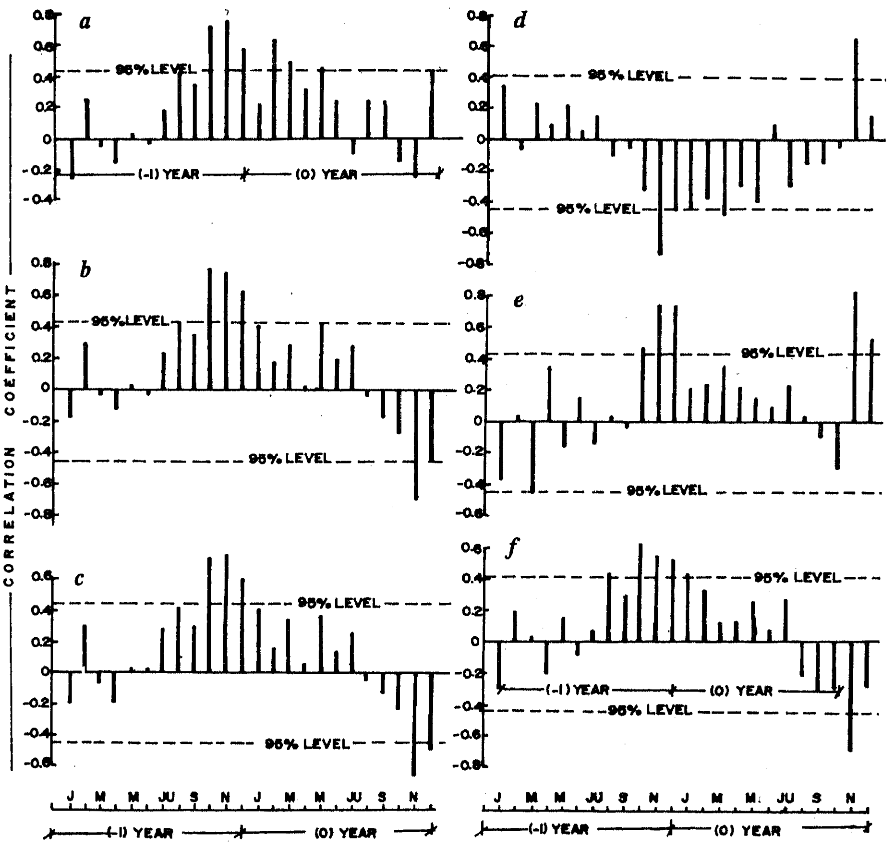

Figure 1. Correlation coefficients between SST and (*a*) R_1, (*b*) R_2, (*c*) R_3, (*d*) DWPJ-A, (*e*), BMBPA-J and (*f*) A-MR500.

Table 1. Sliding window correlation between SST and different parameters

Period	(a)	(b)	(c)	(d)
1950–75	0.11	– 0.20	0.26	0.51
1951–76	0.11	– 0.26	0.31	0.50
1952–77	0.09	– 0.26	0.35	0.46
1953–78	0.14	– 0.30	0.40	0.47
1954–79	0.15	– 0.31	0.41	0.45
1955–80	0.15	– 0.30	0.41	0.45
1956–81	0.15	– 0.37	0.43	0.55
1957–82	0.22	– 0.38	0.64	0.53
1958–83	0.35	– 0.51	0.58	0.55
1959–84	0.33	– 0.53	0.58	0.54
1960–85	0.35	– 0.59	0.64	0.51
1961–86	0.40	– 0.58	0.65	0.50
1962–87	0.57	– 0.66	0.66	0.50
1963–88	0.63	– 0.67	0.65	0.53
1964–89	0.65	– 0.67	0.65	0.53
1965–90	0.64	– 0.67	0.66	0.54
1966–91	0.67	– 0.65	–	0.47

(a) Correlation between October SST of the previous year in 0–5°N; 80–85°E and R_2.
(b) Correlation between November SST of the previous year in the same area and DWPJ-A.
(c) Correlation between November SST of the previous year in the above area and BMBPA-J.
(d) Correlation between April SST in the north Australia–Indonesia region (5–15°S; 120–160°E) and R_2.

the slowly varying planetary circulation and SST anomalies in the equatorial region[11] the observed relationship between October SSTA and A-MR500 is justified. More northward (southward) displacement of the 500 mb ridge position is a precursor for strong (weak) monsoon. In view of the above discussion, the observed strong correlation between October and November SSTA (– 1 year) and monsoon rainfall appears to be reasonable.

It is suggested that the SST in the EEIO may be included in the operational forecast model of India Meteorological Department.

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On the age and fauna of beachrock of Kegaon Coast, Uran, Maharashtra

Outcrops of beachrock occur as isolated patches all along the coast of Maharashtra and comprise conglomerates, shell limestone bands and consolidated dune sand designated as 'karal'. The karal has been dated and found to range from Middle to Late Holocene in age[1–4]. The beachrock of Kegaon Coast of Uran forms the northernmost outcrop of the Raigad district. We aim to comment here on the age and paleoenvironment of the karal of Uran. It is well exposed in the intertidal and supratidal zone, stretching over 500 m and resting on highly fractured and well-jointed amygdular basalt. The karal has an uneven contact with basalt and exhibits varying thickness (Figure 1). The lithologic section studied shows development of approximately 3.5 m thick sediments with a basal conglomeritic zone followed by laminated shell limestone bands and dune sand (Figure 2).

Survey using theodolite was carried out at a location (18°52′58″N and 72°54′48″E) to determine the position of the beachrock with respect to the present MSL (Figure 3). The bases of conglomerate and dune sand lie at 1.5 m and 2.5 m above MSL respectively. However, the thickness of the beachrock varies from place to place. A section at Uran (18°50′25″N and 72°50′50″E) illustrated by Agarwal and Guzder[1] appears to be 4 m thick, while Kale *et al.*[5] gave a range of thickness from 1.5 m to 2 m for the beachrock and 0.9 m to 1.5 m for the dune sand exposed at a location having coordinates 18°52′30″N and 72°54′56″E.

The beachrock is compact, fairly well consolidated towards the bottom and fragile towards the top. The fauna recovered from the beachrock comprises mollusca, foraminifera, broken fragments of bryozoa, corals, etc. The shell limestone in particular, is rich in gastropods species,

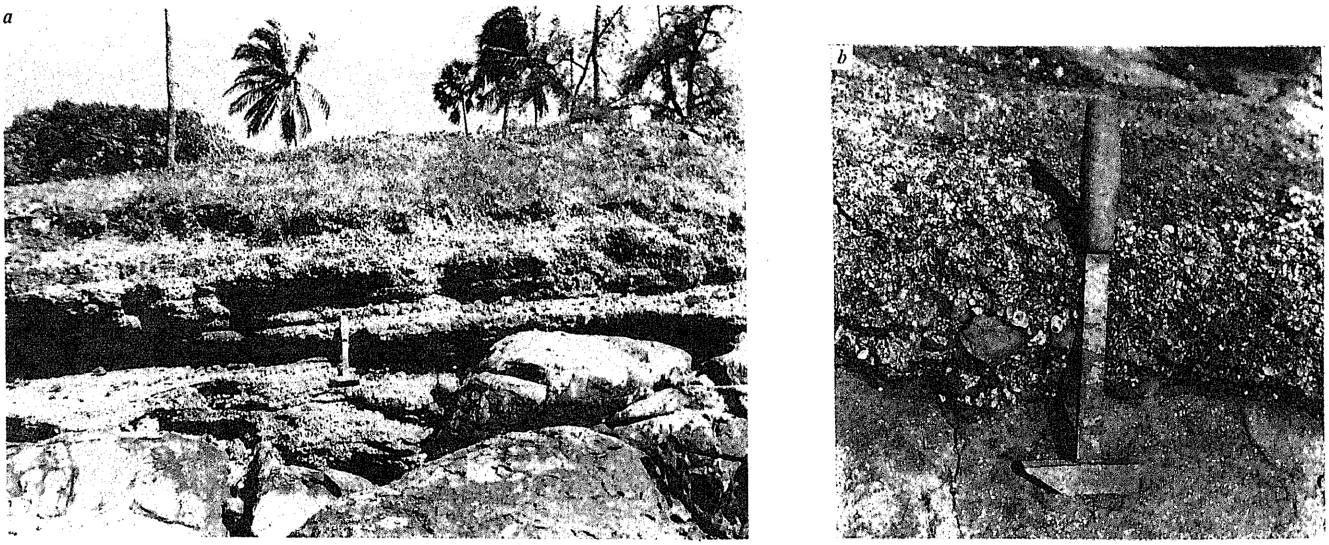

Figure 1. *a*, Outcrop of beachrock along Kegaon Coast, Uran. *b*, Contact between the basalt and beachrock.

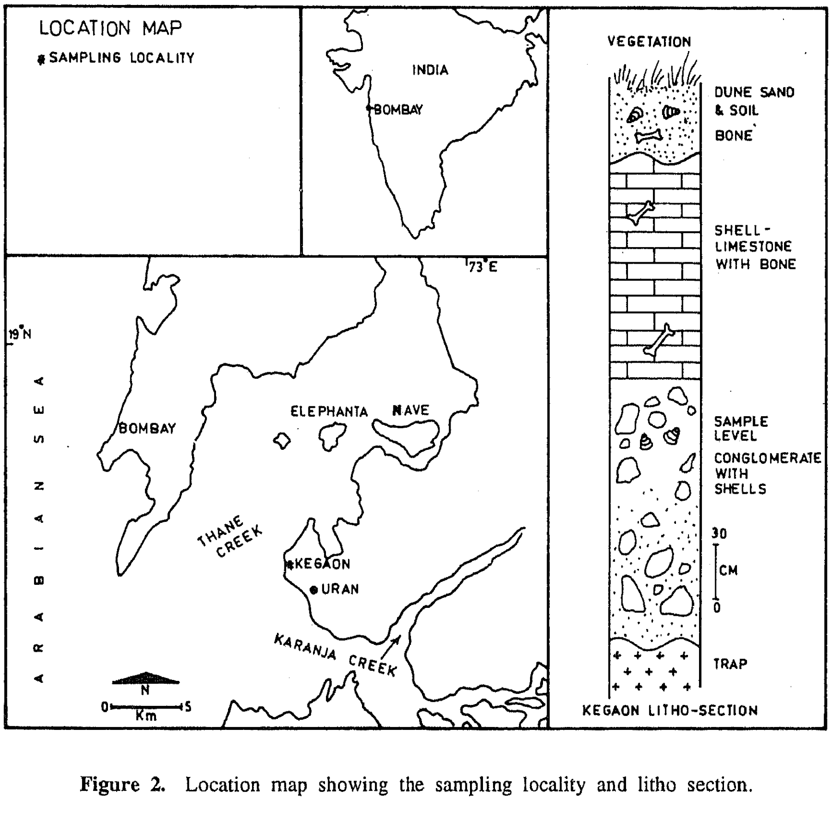

Figure 2. Location map showing the sampling locality and litho section.

viz. *Nerita (Theliostyla) oryzarum* (Recluse), *Trochus (Infunidibulum) radiatus* (Gmelin), *Clypeomorus moniliferous* (Kienar), *Cerithidea cingulata* (Gmelin), *Conus* sp., *Thias tissoti* (Petit), *Cantharus* sp., etc. The foraminifera belong to genera *Ammonia*, *Elphidium*, *Eponides*, *Bolivina*, *Brazalina*, *Siphogenerina*, etc. Fossils are rare in dune sand. Isolated mammalian bones and teeth occur at the contact of dune sand and shell limestone and in the upper part of the shell limestone band (Figure 2).

Being aragonitic, gastropod species *Nerita (Theliostyla) oryzarum* (Recluse) is used for carbon dating. The scanning electron microscope studies of the shell wall revealed undisturbed pattern of acicular aragonitic crystals (Figure 4 *a*), thereby ruling out any possibility of post-depositional leaching which otherwise would have affected the accuracy of the ^{14}C date. The measured radiocarbon age of the sample (BS 1204) is 1170 ± 90 years BP. This age when corrected using the radiocarbon age calibration programme for marine data base of Struiver and Braziunas[6] gives the calibrated age as 700 ± 70 years BP. A radiocarbon date BP 600 ± 80 for a fossil mangrove wood (BS 1051) from mud beach at Rewas, south of present locality across the Dharmtar Creek[7] revealed a relatively younger age for the sediments in the vicinity.

Formation of beachrock is a regional phenomenon and its distribution is related to time and altitude with respect to present day MSL. For Holocene, the global trends of elevation versus time plot indicate that older the strata, the higher or deeper is the position with respect to zero elevation[8]. Interestingly, Kale *et al.*[5] on the basis of fluorine/phosphate ratio of a mammalian bone assigned Late Pleistocene age and suggested that the beachrock of Uran is older than that occurring south of Thane Creek. Contrary to this, ^{14}C date obtained from *Nerita* sp. suggests that it is the youngest of beachrocks so

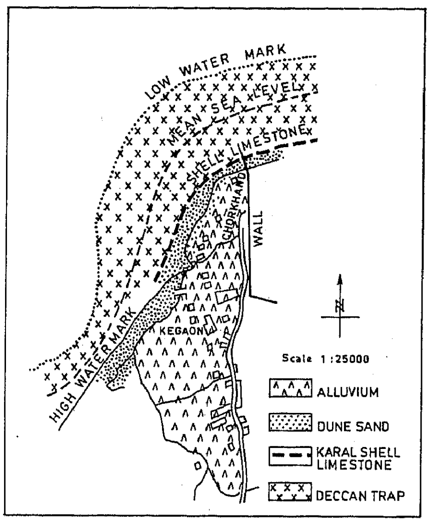

Figure 3. Position of beachrock with respect to main sea level.

far reported from west coast of Maharashtra. Hence, conclusions drawn by Kale *et al.*[5] regarding the Late Pleistocene age for the Uran rock are not tenable as during the Late Pleistocene, globally the sea level was much lower (120 ± 20 m). Therefore, only a localized single isolated patch cannot be older in age while all other available dates are in the range of Mid to Late Holocene.

Thus, the fragmentary mammalian bones seem to be of derived nature, further confirming that definite Late Pleistocene deposits along the west coast of Maharashtra are yet to be recorded[4].

From foregoing discussion it is evident that there existed a higher strand line along the coast of Uran at about 700 ± 70 years BP. Deposition of beachrock commenced with transgressive pulse, resulting into formation of conglomerate comprising angular to subrounded pebbles and cobbles of basalts, molluscan shell fragments and other bioclasts. These are further lithified by acicular aragonitic cement (Figure 4 *b*). Thin aragonitic coatings, observed around the sediment grains as revealed under SEM, indicate deposition in the lower intertidal zone[9]. Due to subsequent fall in sea-level, conglomerate and shell limestone were covered by dune sand. The upper portion of shell limestone and dune sand is highly fragile and yet to be lithified. However, a little amount of calcitic cement has developed due to influence of meteoric water (Figure 4 *c*).

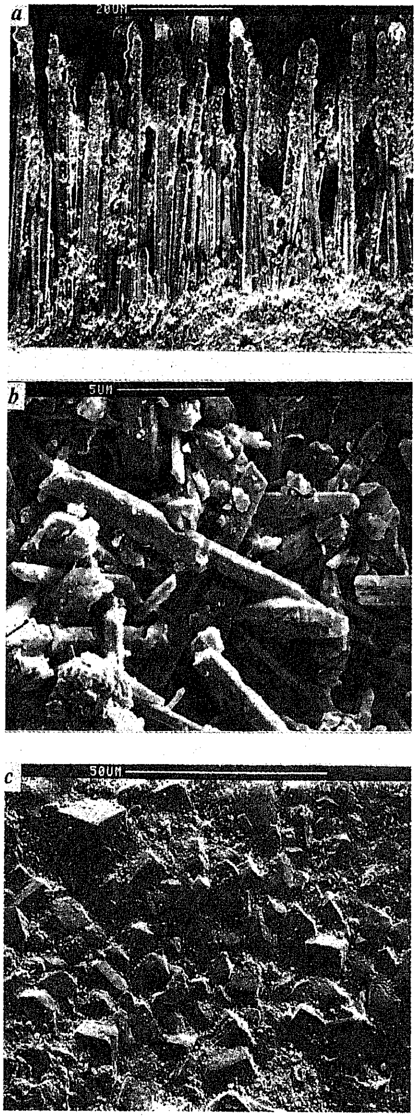

Figure 4. *a*, SEM photograph of the shell wall of *Nerita (Theliostyla) oryzarum* showing undisturbed acicular aragonitic crystals. *b*, SEM photograph showing acicular aragonitic cement as thin coating around sediment grains. *c*, Interstitial calcitic cement developed due to meteoric water.

Thus, the karal of Uran has been deposited under a Late Holocene transgressive pulse which lasted for a few hundred years only. Presently, the coast of Uran is under active erosion and the karal is subjected to a rapid destruction.

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Parasitic infestation on commercially important sardines

In India, sardines (*Sardinella* spp) constitute the single genus with highest level of production[1]. Such important fishes are frequently encountered with parasitic infestation. But detailed knowledge on the different parasites of sardine is scanty[2–4]. Apart from the oldest records, nothing is known on the parasites that live at the expense of sardines.

During biological observations on the *Sardinella* spp of east coast of India, several sardines with external parasitic infestations were noticed. More than 25 isopod parasites belonging to the genus *Nerocila* were collected from the sardines *Sardinella gibbosa*, *S. albella* and *S. dayi*. Most of the parasites (83%) were attached on the opercular region. On the caudal region of the sardines the *Nerocila* sp. attachment was 17%. About 79% of the parasites attached on the anterior region was only on the left side of the host fish. The position of attachment was a little angular. The appendages in the anterior region of the parasite were curved and pointed to establish a firm grip of the host tissue. The infected area or area of the attachment of the parasite on sardine was devoid of scales and outer dermal layer. Skin lesion was also observed at the site of attachment. The *Nerocila* sp. was found to carry eggs and larvae in the egg pouch on the ventral side of the trunk. An instance of hyperparasitism (Figure 1) was also encountered. In a specimen of *Nerocila* collected from *S. gibbosa*, a copepode parasite belonging to Lernaeoceriformes *Lernaeconicus* sp. was noticed. The anterior part of *Lamaeonicus* sp. was buried inside tissues under the large egg mass on the ventral side of the host.

$$K = \frac{\text{Body weight} - \text{Gonad weight} (\times 100)}{\text{Length}^3}$$

The condition factor calculated for the parasitized sardines (0.38 ± 0.06) and non-parasitized sardines (0.9 ± 0.04) (collected in the same catch, same season and length range 12–14 cm TL) indicated that the parasitic infestation has affected the *K* value. The reduction in *K* value in the parasitized fishes has been ascribed because of the retardation of the gonadal development due to parasitic infection. This was further reinforced by the maturity studies carried out on the gonads of parasitized and non-parasitized sardines. In all the parasitized sardines the testis and ovary are in early stages of development and in some cases gonadal differentiation had not taken place. But in non-parasitized sardines the testis and ovary had attained maturing conditions just prior to spawning.

In addition to the isopod parasites, Lernaeoceriformes a copepod, parasite, *Peroderma sardinellae*[5] were also collected in large numbers from *S. albella*, and *S. sirm*. *P. sardinellae* was found to be a tissue borer making holes (2–4 mm diameter, depth up to inner organs) on the host body. The entire body of the parasite was buried (Figure 2) inside the bore except the egg sac. The bore was mostly (90%) in slanting position. There was no indication of scales on the bore and scales at the margin of the bore showed signs of drilling, with concave erroded margin. The parasite was covered by chitinous jacket and had anastomosing rhizoid structures on their antero-lateral side to absorb nourishment from the host. The condition factor calculated for *P. sardinellae*-infected fish was (0.3 ± 0.03) low when compared with non-parasitized fishes (0.9 ± 0.04). *P. sardinellae*-infected fishes had retarded maturity conditions when compared with non-parasitized fishes in similar lengths group. In the parasitized sardines the length range from 11 to 14 cm TL, the gonadal maturation has not taken place. The gonads remained as immature testis and ovary. But in the non-parasitized fishes the gonads developed prespawning conditions both in the males and females.

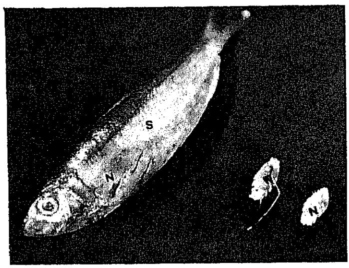

Figure 1. Sardines (S) parasitized by the isopod *Nerocila* sp. (N). An isolated *Nerocila* sp. infected by *Lernaeonicus* sp. showing hyperparasitism.

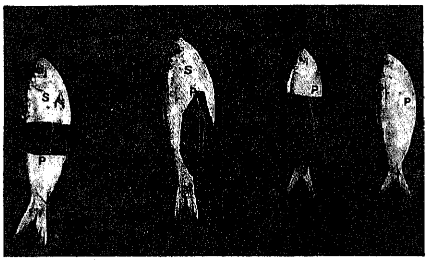

Figure 2. Sardines (S) parasitized by *Peroderma sardinellae*. The parasites are buried inside the host tissue, only the egg strings are seen outside.

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GENERAL ARTICLE

Transgenic plants and biosafety concerns in India

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The application of genetically modified plants in agriculture would be one of the many scientific inputs to accomplish increased agricultural production. The concerns are the risks and the magnitude of the consequences to the environment. Interestingly after scientifically generating data on safety, certain countries have approved the large-scale use of some transgenic crops. India started its field experiments in open environment using transgenics from 1995. The Government of India set out the rules and regulations for handling the genetically modified organisms (GMOs) which include transgenic plants, in 1989 through its Environment Protection Act, 1986 (EPA). The rules narrate procedures for conducting R&D experiments using GMOs as well as for large-scale applications, using them. Following the clearance under EPA, the transgenic plants are eligible for evaluation in the All India Coordinated Trial (AICT) procedures of the Ministry of Agriculture and Cooperation (MOA) for release by the latter for commercial applications in Indian agriculture.

THE protection and preservation of the environment in India is vested upon the government. The Central Government had enacted the environment protection laws from the Ministry of Environment and Forests (MOEF) from time to time to preserve the environment and to protect it from damage. Protection of environment includes assessment of the effects of substances or organisms to animal and plant health besides their effects both on the ecosystem and on the surroundings. In pursuance of the aims and objectives of preserving the environment, the Central Government from the MOEF had promulgated in December 1989 the rules and procedures for the manufacture, import, use, research and release of genetically modified organisms (GMOs) as well as products made by the use of such organisms. These orders were necessary, as the organisms and substances made by the use of genetic engineering hold potential risks to human and the environment. Therefore, without adequately assessing the environmental risks, their use or release into the open environment was considered unsafe and unwise. The intentions of such rules and regulations had never been to hinder or retard the progress of science and technology but to ensure adequately that the use of such products or life forms were safe to the environment, and beneficial to the human beings.

In this paper the biosafety concerns from the use of *transgenic plants* are discussed. Together with this, the existing rules and regulations for using such plants are also briefly enumerated.

Background information and historical highlights

India has realized that to sharply increase the food, feed and fibre production from the current level, there is no escape from use of modern biotechnology. Modern biotechnology includes isolation and purification of target DNA, RNA and protein (enzymes, hormones); cloning and amplification of genes; construction of vectors and hosts; standardization of cell or tissue culture techniques; optimization of downstream processing methods for the handling and stabilization of either the gene products or the GMOs. Genetically modified organisms are the organisms which have been modified through human intervention by recombinant DNA technology, and which are either self-replicating species or which replicate via a host organism, or which have reproductive capabilities. GMOs *exclude* organisms produced by breeding as well as organisms produced by the intraorganism rearrangement of genetic materials by physical methods or by chemical means.

India is spread over 3,287,263 sq km and is the 7th largest country in the world. Its population was 885 million in mid 1993 (16% of global population) which is expected to rise to 1015 million by 2000 AD, and 2530 million by 2050 AD. More agricultural yield is,

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Views expressed in this paper are those of the author and they do not necessarily express the views of the organization to which he belongs.

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therefore, absolutely necessary for enabling to feed the growing population. The food grains production in India is on the rise; it increased from 176.4 million metric tonnes (mmt) in 1990–91 to 189.8 mmt during 1994–95 (ref. 1). However the increases are almost proportional to the rise in the population and therefore the per capita consumption has remained almost static during the last one decade. In order to maintain the current growth rate in agricultural crops as well as to improve upon the present yields further, various means are to be adopted. Use of modern biotechnology, involving the application of genetically modified plants, would be one of the many scientific inputs to agriculture to accomplish increased production. However, our unfamiliarity with these new inventions has made us more than concerned and alert to deal with them. This situation is true not only in India but also in all the developing countries as well as in the developed nations.

Current biosafety concerns

The main concerns are the right assessment of the magnitude of the consequences from the use of genetically modified plants to the habitat including human and animals, the flora and fauna, and the environment. Certain hereditary traits are targeted for transfer into the transgenic plants by using recombinant DNA technology as discussed subsequently in this text. The concerns[2] are that the chemical herbicide-resistant plants may confer new properties to the near-relative wild plants to make them also resistant to herbicides by transferring the resistant pollens to them and thus may transform them into plants, resistant to the currently used chemical herbicides. This situation would not be controllable by the known chemical herbicides and therefore agricultural production would become more expensive; viral disease-resistant plants may provide opportunities to evolve newer virulent strains of plant viruses by recombination between the viral genes contained in the modified transgenic plants and other infecting viruses against which the target plants are not protected; insect-resistant plants (for example, developed by incorporating specific *Bacillus thuringiensis* (*Bt*) genes, coding for proteins toxic to pests, into plants) may create situations of fast loosing the biopesticide value of the gene products (and therefore the genes),

Table 1. Transgenic crops approved in USA for commercial use

Product	Genetically altered traits	Company	Year of approval and product name, if any
Tomato	Delayed ripening	Calgene	1994, Flaver Savr™
	Delayed ripening	DNA Plant Tech.	1995, Endless Summer™
	Delayed ripening	Monsanto	1995
	Thicker skin and altered pectin content	Zeneca/Petaseed	1995
Cotton	*Bt* gene incorporated plants (ballworm and budworm resistant)	Monsanto	1995, Bollgard™
	Resistant to bromoxynil	Calgene	1995, BXN Cotton™
	Resistant to glyphosate	Monsanto	1996, Round up ready™
	Resistant to sulphomoyl urea	Dupont	1996
Soybean	Resistant to glyphosate	Monsanto	1995, Round up ready™
Potato	*Bt* gene incorporated (Colorado potato beetle resistant)	Monsanto	1995, New Leaf™
	Insect resistant (*Bt* gene incorporated)	Monsanto	1996
Maize	*Bt* gene incorporated (resistant to cornborer)	Ciba-Geigy	1995, Maximizer™
	Resistant glufosinate	Devalb	1996
	Resistant to glufosinate	Agro Evo	1996, Liberty Link™
	Male sterility	Plant Genetic Sys.	1996
	Bt gene incorporated (resistant to cornborer)	Monsanto	1996, Yeild Gard™
	Bt gene incorporated (resistant to cornborer)	Northrup King	1996
Rapeseed/ canola	Altered oil composition (high lauric acid)	Calgene	1995, Laurical™
Squash	Resistant to viruses	Asgrow	1995, Freedom II™

TM = Trademark.

as resistant insects are expected to be developed faster than is surmised, by excessively deploying such transgenic plants in the open environment. Besides, there are also concerns that the use of some of these plants and their products in the human food chain could prove allergic to some people[3].

Current global scenario

Interestingly, after conducting risk assessments and after the evaluation of their hazards by own methods, certain countries have already permitted the use of some transgenic plants in their commercial agriculture. China was the first country to introduce in the early 1990s the viral-resistant tobacco and later the viral-resistant tomato for commercial use[4]. Subsequently in May 1994, Calgene Inc., USA introduced its Flavr Savr^{TM} delayed ripening tomato[2,4] for commercial use. By the end of May 1996, seven transgenic crops involving 10 traits, using 19 processes were approved for commercial use[4] in USA (Table 1).

According to one estimate, up to the end of December 1995, nearly 3650 field trials had been conducted the world over[4], and as already indicated above, several plants have been authorized for commercial exploitation in USA. Information about approval from other countries was not available but it is expected that several approvals might have been accorded in Canada, China, Japan, Australia, Argentina, Mexico and certain European countries. It is estimated by the author that currently in 1996 the world over about 4 million acres of land are used for genetically engineered crops in commercial agriculture and over 75% of the land use for transgenes is in the USA alone followed by Canada (15%) and other countries (10%) (ref. 5).

Countries are permitting the commercial use of transgenic plants obviously because they are satisfied about the biosafety aspects from their use. The approved plants must have also provided additional economic advantage in experimental trials by manifesting the properties imparted upon them through recombinant DNA technology, e.g. herbicide tolerance, increased insect resistance, specific viral disease resistance, etc.; it is too early, however, to be assured with certainty that in every case of large-scale use, the economic advantages are real and sustainable, till the world community uses these plants extensively over long periods. Already concerns have been raised on the utility of *Bt* cotton in USA[6]. However, the current tempo is to maximize the use of transgenic plants at least in certain industrialized countries. The use of transgenic plants with these imparted agronomic traits is therefore anticipated to increase, and may contribute to the economy a global market of between US$ 2 and 3 billion in 2000 AD and $ 6 billion in 2005 AD (ref. 4).

Globally at least 64 different commercially important species of plants have been utilized for incorporating transgenic traits; important among these are maize, soybean, cotton, tomato, potato, alfalfa, petunia, rape/mustard, rice, wheat, beet, barley, Bengal gram, cabbage and tobacco. The traits that have been targeted for genetic acquisitions by the plants could be classified broadly as 6 only (Table 2), and that the tolerance to herbicides head the list[2,4].

Field experiments using transgenics in India

In India, field experiments using transgenic plants began in 1995 when the first permit was issued to M/s Pro Agro Ltd., New Delhi in September 1994, to conduct field trials (in contained and controlled environment) at Gurgaon (Haryana) using transgenic rape seeds (*Brassica juncea*) containing Barstar genes (producing male sterile seeds) and Barnase genes (sterility restorer). Subsequently up to August 1996, permits have been issued for limited field trials using *Bt* gene incorporated tomato and cotton[7]. In these experiments the commercial interests had been to develop improved local varieties from the transgenics (by back crossing etc.). The primary concerns from the regulatory authorities were, however, to find answers to the questions of risks and environmental safety.

Indian rules and regulations on GMOs including transgenic plant varieties

The rules and regulations for the manufacture, use, export, import and storage of genetically engineered organisms have been notified in the official Gazette of the Government of India by the MOEF through their Notification No. 621 dated the 5th December 1989. The notification was issued by the Government by virtue of powers conferred upon it through the EPA, 1986. Any violation and noncompliance including nonreporting of the activities in this area would attract the punitive actions provided under the EPA.

The Department of Biotechnology (DBT) of the Ministry of Science and Technology implements the R&D experiments utilizing GMOs and rDNA products, while the MOEF implements the large-scale commercial use and deployment of these.

Table 2. Genetic transfer of traits in transgenic plants by recombinant DNA technology

- Herbicide tolerance.
- Insect resistance
- Viral disease tolerance
- Fungal disease tolerance
- Product quality improvements
- Others (production of speciality metabolites/ chemicals, incorporation of marker genes, stress-resistance properties, etc.)

Indian structure for R&D experiments using GMOs

For R&D experiments, the structures enumerated in the above-mentioned notification are that it directs creation of various committees with set objectives for observing and implementing biosafety guidelines. The biosafety guidelines, available from DBT[8] were prepared by the DBT in 1990 and subsequently revised in 1994. Every organization involved in R&D using GMOs is required to setup its Institutional Biosafety Committee (IBSC), which has a DBT nominee and which is the nodal point for interaction with the Government within the institution/organization. The Review Committee for Genetic Manipulation (RCGM) is a national committee functioning under the DBT and has the functions of reviewing all the approval of ongoing R&D projects on GMOs, undertakes field visits of sites of experimental facilities and issues clearance for import/export of etiologic agents, vectors, germplasms, organelle, etc. needed for experimental working, training and research. Based on the recommendation of the RCGM, trial permits are issued by the DBT. Experiments are monitored by the RCGM besides the IBSC; in addition, the concerned State Biotechnology Co-ordination Committees (SBCC) of each State and the District Level Committees (DLC) of each district are also involved in the inspection and monitoring of the experiments at the field sites.

Indian structure for large-scale applications using GMOs

The Genetic Engineering Approval Committee (GEAC) of the MOEF is the interministerial committee with subject specialists as Members, and is the Competent Authority for any large-scale use of GMOs. The MOEF would issue the authorization to applicants on the basis of approvals accorded by the GEAC. As in the R&D trials, the large-scale use authorized by the GEAC also involves the watch on field applications of GMOs by the SBCCs and the DLCs. The GEAC would also involve the user Ministries as well as the other regulatory authorities (for example, for Drugs and Pharmaceuticals, the Directorate General of Health Services are consulted; for other items the concerned administrative agencies are consulted) before authorizing any commercial use of GMOs and rDNA products. Eventually, it is the concerned Administrative Ministries which take over after the clearance under EPA is accorded by the MOEF.

Field trial methods using transgenic plants

Planned field experiments using transgenic plants are permitted only after a stepwise evaluation of the developmental process from lab scale to the growth chambers and greenhouse conditions are thoroughly evaluated. The data for evaluation may be generated in India or elsewhere. The documentation requires full characterization and description including the sequence of the target gene and its promoter sequence as well as the regulatory mechanism utilized in the expression casette, diagram of the expression casette to describe fully the marker genes used, the description of the restriction sites related to specific endonuclease, the cell lines used for shuttling and amplification of the expression casette, the method of construction of the target gene along with all the sequences added or deleted, the extent of target gene amplification into the host genome and the description of the amino acids sequence being transcribed by the target gene as well as the marker genes. Lab data are insisted upon to prove beyond doubt that the translated gene products as well as the plants are safe to the environment and the human beings. If such data are not available, these are asked for to be generated.

Certain criteria are insisted upon which are to be followed while conducting field trials in small scale (R&D experiments) or in large-scale as indicated below.

1. To maintain at least the minimum isolation distance recommended for raising 'Foundation Seeds' all around the transgenic plants. Within the isolation distances noncompatible crops could be grown. For the details of the isolation distances for various crops, the publication of the Central Seed Certification Board of the Department of Agriculture and Cooperation, MOA for foundation seeds is to be followed[9].
2. Beyond the isolation distance, a few rows of the nontransgenic plants of the same crop to serve as a pollen trap may be grown.
3. In order to measure the distance of pollen escape from the target plants, within the isolation distance at intervals of 1 to 5 m nontransgenic plants can be grown; the seeds set at different isolation distances are then collected individually and separately aggregated, examined for the transgenic traits and the marker gene traits to work out and assess the extent and the distance of pollen transfer/flow from the target experimental sites.
4. Safety tests include the generation of data on the elucidation of genetic markers, host range requirements for vegetative growth, persistence and stability in small plots and experimental field trials for at least two years.
5. All the vegetative plants and left over seeds are to be destroyed by burning after the experiments.
6. The land may be left fallow next year after the experiments are over and the plants, if any, emerging in the soil from the seeds of last year may be destroyed.
7. The experimental field may be visited by the company authorized personnel only, and all records of visits are to be maintained.
8. Full account of transgenic seeds produced are to

be kept and no transgenic seeds be transacted or further propagated without authorization.

Discussion

The decisions for the application of transgenic plants in commercial agriculture are intimately connected with the concerns of safety among all cross sections of society. Decreased familiarity about the risks associated with their use aggravates the situation further. The concerns assume great significance particularly for applications in agriculture, as the products would be parts of human and animal food chain. It is for these primary reasons that the questions of safety from the application of transgenic plants are being passionately posed by different countries in different fora.

In order to be assured about all aspects of safety from the use of new technologies, it is surmised that certain steps taken sequentially, and answers found on sound step-by-step scientific assessment would resolve all the issues concerning safety[10]. It is mentioned in this connection that steps should be taken gradually and steadily without showing an iota of hurry and rush to reinstate the process of confidence building among all sectors of human community. In all initial assessments in the open environment, contained experiments must first be carried out. The purpose of containment is to ensure fool-proof methods of maintaining control over the spread of the GMOs in the open environment. Further, it is to reduce the exposure of GMOs to the personnel handling them, particularly in cases where the GMOs are associated with any human allergens. The following steps are logical, for which answers from different angles should be found in the registration document, based on which it would be possible to decide about safety and associated risks.

a) To systematically *identify the hazards* from the use of transgenic plants.
b) To *assess the risks* from each identified hazard by mounting convincing experiments and by collecting scientifically convincing data.
c) To *manage the risks* by applying logically valid strategies which would, inter-alia, include methods and procedures for minimizing risks.

In order to systematically identify the hazards and to assess the risks, information on the plants with transgenic traits should be generated on the following aspects.

1. Characteristics of the *donor organisms* providing the target nucleic acids. These may include the following:
a) Name of the donor organism with its identification characteristics with relevant reference to published information, if any.
b) Pathogenicity and toxicity characteristics to plants and animals.
c) Allergenicity characteristics to human along with identification of the allergenic substances, wherever possible.
d) The geographical origin of the organism, its distribution pattern and survival mechanism.
e) The method of transfer of its genetic materials to other organisms.
2. Characteristics of the *vectors* used: These may include the following:
a) The origin, identity and habitat of the vectors used.
b) The sequence, frequency of mobilization, specificity and marker genes if any, present in the vectors.
c) The abilities of the vectors to get established in other hosts; the hosts are also to be specified.
3. Characteristics of the *transgenic inserts*: These may include the following:
a) The specific functions coded by the inserted nucleic acid stretches including the marker gene inserts.
b) The expression of the nucleic acid products and their activities/properties.
c) The toxicity of the expression products on the host plant, if any.
d) The toxicity and allergenicity of the nucleic acid products to human and animals.
4. Characteristics of the *transgenic plants*: These may include the following:
a) Methods of detection of the transgenic plant in the environment.
b) Methods of detection and characterization of the escaped transgenic traits in the environment.
c) Toxicity and pathogenicity of the transgenic plants and their fruits to other plants in the ecosystem and the environment.
d) Possibility of and the extent of transgenic pollen escape and pollen transfer to wild near relatives, and the consequences to the environment.
e) Pathogenicity, toxicity and allergenicity of the transgenic plants and their fruits to human and animals.
f) Changes in the soil microflora due to cultivation of transgenic plants.

Information on many of the above questions may not yet be available. Therefore appropriate new experiments would have to be designed to gather data. Unfortunately, universal model experiments have not yet been designed for collecting data to reply to all the questions posed above. In seemingly controversial situations, the next best alternatives are to be found out after considerable scientific debate in the country. As regards generating information on toxicity and allergenicity, standard protocols are to be devised for collecting data utilizing small adult lab animals. The toxicity data should include acute as well as chronic toxicity studies; in doubtful situations genotoxicity, teratogenicity, neurotoxicity and immunotoxicity data should also be generated in small lab animals. Chronic toxicity data may also be generated using large locally available mammals like goats or sheeps or cows or dogs for about 8 to 12 months, but not less than 3 months. Utility of data from these animals may be debated for reasons of nonuniformity of genetic makeup of these animals as they are outbred, they are ruminants and are therefore more distantly related to human species than monkeys are. It is surmised, however, that the feeding data generated on these animals found in local environment would help in the resolution of local concerns about the risks from the long-term use of transgenic plants, entering directly or indirectly into the human food chain. Safety information generated on animals abundantly available in local environment and closely linked with human habitat is more ensuring to human than the extrapolated assurances drawn from data on distantly related varieties of small lab animals. Similarly, feeding data generated on some common avian species like sparrows, parrots or pigeons, which are found in the local ecosystem, is expected to put to rest the anticipated fear of creation of disturbances in the environment from the use of GMOs.

Once the questions regarding identifying the hazards and assessing the risks from various angles are resolved, it would be easier to *manage the risks* by applying logically valid strategies. The strategies are to be commensurate with the risks identified. The risk-management strategies for the release of transgenic plants may take into account the following:

a) Spacial separation for isolation, for preventing reproduction/fertilization and seed setting.
b) Biological prevention of flowering by making use of sterility properties, etc.
c) Human intervention for the removal of reproductive structures of flowers.
d) Controlling the reproductive structures of transgenic plants like the seeds and the plant propagules from unaccounted spread.
e) Controlling and destroying volunteer plants from the experimental field.
f) To take into account the proximity to human activity in case the transgenic plants have allergenic properties to human and animals.
g) Appropriate training of field personnel responsible for handling the transgenic plants.
h) Plans for handling unexpected events.
i) Documentation of previous published information, if any, including any documented evidence of effects of release to ecosystem.

For the assessment of hazards and risks, and for the management of risks the recombinant DNA safety guidelines of the DBT[8] should be strictly adhered to. In order to enable the GEAC of the Government of India in the MOEF about the proper assessment of risks and hazards from the use of transgenic plants before permitting their large-scale release, information on the lines discussed above is required to be submitted in a format which was devised for the purpose[11] by the government some years ago. The format needs to be updated.

Based on the assessment of the above information, prepared by the applicant in the form of a document which could be called the Registration Document, the GEAC would decide if clearance for commercial use would be permitted for specific transgenic plants. Up to the present time, however, no transgenic plant variety has yet been cleared by the Government of India for large-scale commercial use.

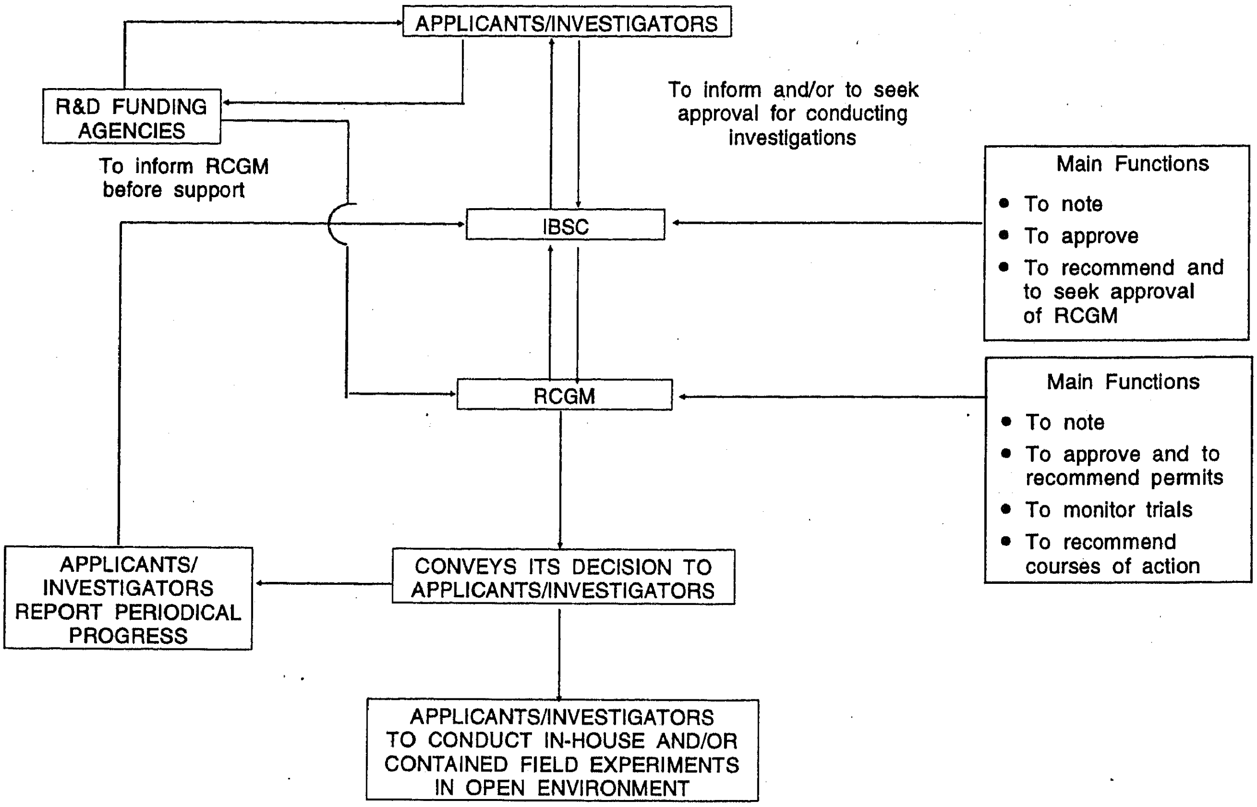
The GEAC clearance is only from the environmental angle under the EPA. Following the GEAC clearance, the applicants are to seek the clearance of the MOA, who in turn would then install the procedures of AICT through the system of Indian Council of Agricultural Research (ICAR), New Delhi. The final clearance of transgenic agricultural crops is accorded by the MOA through its well-established wing of assessment. Figure 1 shows schematically how a transgenic variety is to be eventually cleared by the Government for extensive field use, starting from research stage. It could be seen therefrom that the GEAC may, based on the information generated elsewhere by the applicant, or through the RCGM mechanism in India, accord approval to the applicant to proceed further for initiating the All India Coordinated Trial (AICT).

The experience gathered so far in India is indicative of a minimum time requirement of 3 years for generating information for submission to the GEAC for environmental clearances of GMOs, if proceeded through the IBSC and the RCGM mechanisms. Subsequently, the AICT would also take 1 to 3 years before enough data is generated for the MOA to enable the latter to decide on varietal clearance. In other words, a minimum of 4 to 6 years are required for transgenic plants to get entry into the Indian Commercial Agriculture.

Concluding remarks

The hazards and risks are to be assessed based on up-to-date current knowledge on the transgenic plants. The common elements and principles drawn from the experiences of other countries would go a long way in our understanding and in confidence building, although in certain situations experiments based on local conditions would have to be devised and local data generated. It has to be kept in mind that unfamiliarity with transgenics would not necessarily mean that they are *per se* unsafe. Our monitoring of the transgenics shall have to be intensified from simple visits and simple in-place observations of transgenic plants in fields by the expert team, to designing extensive research programmes to seek answers to specific safety questions. This would also need to broaden the knowledge base of the expert

a) Research use


APPLICANTS/INVESTIGATORS
R&D FUNDING AGENCIES
To inform RCGM before support
To inform and/or to seek approval for conducting investigations
IBSC
Main Functions
• To note
• To approve
• To recommend and to seek approval of RCGM
RCGM
Main Functions
• To note
• To approve and to recommend permits
• To monitor trials
• To recommend courses of action
APPLICANTS/ INVESTIGATORS REPORT PERIODICAL PROGRESS
CONVEYS ITS DECISION TO APPLICANTS/INVESTIGATORS
APPLICANTS/INVESTIGATORS TO CONDUCT IN-HOUSE AND/OR CONTAINED FIELD EXPERIMENTS IN OPEN ENVIRONMENT

b) Large scale commercial agricultural use

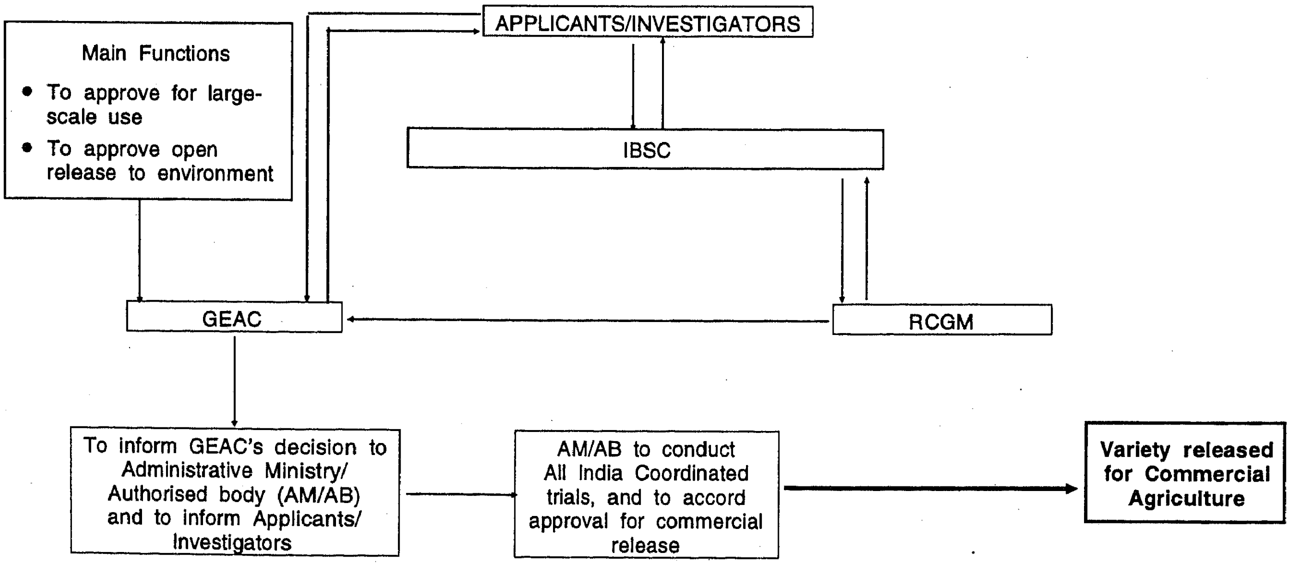

Main Functions
• To approve for large-scale use
• To approve open release to environment
APPLICANTS/INVESTIGATORS
IBSC
GEAC
RCGM
To inform GEAC's decision to Administrative Ministry/ Authorised body (AM/AB) and to inform Applicants/ Investigators
AM/AB to conduct All India Coordinated trials, and to accord approval for commercial release
Variety released for Commercial Agriculture

Figure 1. Handling of transgenic plant varieties for clearance by the Government of India (*a*), for research use and (*b*), for applications in commercial agriculture.

breeders, the plant molecular biologists and above all the science managers and the authorities. The technological area being new, and the world knowledge being yet inadequate, several readjustments and redesigning of strategies to suit local conditions may be required. The future will tell how the countries would go about in this vital new technology area. In the meantime, keeping in view the global developments, specially in the developed world, it is being felt that precious time is running out fast from the hands of many developing countries including India. There has to be a greater sense of urgency in order to develop local capability to enable the generation of right information and to ensure the faster assessments of risks and hazards, and finally to decide on commercial applications quicker. In deciding commercial applications however, a cautious approach would be more desirable than is presently surmised globally in certain countries. A cautious approach is not to foster the creation of unscientific new barriers against the use of GMOs, but to bring in more transparency in their introduction in the open environment. From Indian experience, it appears that the first transgenic plant may get approved for use in commercial agriculture not before 1998.

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REVIEW ARTICLE

How do proteins fold?

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Understanding the mechanism by which an unfolded polypeptide chain folds to its unique, functional structure is a primary unsolved problem in biochemistry. Fundamental advances towards understanding how proteins fold have come from kinetic studies. Kinetic studies allow the dissection of the folding pathway of a protein into individual steps that are defined by partially-structured folding intermediates. Improvements in both the structural and temporal resolution of physical methods that are used to monitor the folding process, as well as the development of new methodologies, are now making it possible to obtain detailed structural information on protein folding pathways. The protein engineering methodology has been particularly useful in characterizing the structures of folding intermediates as well as the transition state of folding. Several characteristics of protein folding pathways have begun to emerge as general features for the folding of many different proteins. Progress in our understanding of how structure develops during folding is reviewed here.

PROTEINS fold to highly intricate and specific structures following their synthesis on ribosomes. The folding of nascent polypeptide chains to their native structures is an essential step in decoding genetic information to cellular activities, because only the folded conformation of a protein is functional. In contrast to our knowledge on transcription and translation as initial steps in information transfer in the cell, little is known about how proteins fold. Obtaining an understanding of the principles governing the formation of a fully-folded native protein from its unfolded state forms the basis of 'the protein folding problem'.

The earliest studies on protein folding date back to the 1920s, when Anson and Mirsky reported, for the first time, that the denaturation of many proteins is reversible[1,2]. This observation was extended by others[3,4], but it was not until 1961 that it was convincingly proven that the three-dimensional structure of a protein is dictated solely by its primary sequence[5,6]. In their classic work on bovine pancreatic ribonuclease A (RNase A), Anfinsen and his colleagues showed that reduced, denatured RNase A refolds spontaneously to its native conformation *in vitro* with the restoration of enzymatic activity as well as formation of its four native disulphide bridges, in the absence of any external assistance[7]. Since this landmark experiment of Anfinsen and his colleagues, research on protein folding has been proceeding at an explosive rate in many laboratories worldwide. Many excellent articles and books have reviewed different aspects of the protein folding problem[8–44]. This review will discuss the results of recent experimental studies that utilized kinetic methods, and will place special emphasis on how structure develops.

A review on protein folding remains incomplete without a discussion of the possible practical applications of protein folding studies[11,45]: (i) Virtually any foreign gene can be expressed in a host bacterium or a cell line using recombinant DNA technology. The expression of the recombinant gene often leads to the production of the protein in an inactive insoluble form, known as inclusion bodies[46]. Recovery of the active forms of these recombinant proteins from their inactive forms requires a good practical understanding of protein folding. (ii) Recombinant DNA techniques and modern peptide synthesis techniques permit the synthesis of large polypeptide chains of any desired sequence. Since the function of a protein is dictated by its three-dimensional structure, it is necessary to know, *a priori*, how a specific amino acid sequence will fold once synthesized. Though reports of designed amino acid sequences which adopt intended folds and possess physical properties similar to those of natural proteins are appearing with increasing frequency[40,47–50], designing proteins with novel biological functions still remains a major challenge. (iii) Although recombinant DNA techniques can be used to modify, at will, the amino acid sequence of an existing protein, the ability to do such protein-engineering in a rational manner depends on first gaining an understanding of how changing the sequence of a protein will alter its structure. (iv) Many diseases, including cystic fibrosis, scurvy, scrapie and Alzheimer's disease, are caused by mutations in essential cellular proteins leading to aggregation or misfolding of the proteins[51–53]. Understanding the mechanisms of these diseases and their subsequent treatment require an understanding of how the relevant proteins fold or misfold. (v) The progress of many genome projects has led to the availability of amino-acid sequences of thousands of proteins. To understand what the roles of many of these proteins in the cell are, it is important to obtain structural information on these proteins. Experimental methods of structure determination (X-ray crystallography and NMR) are slow, and are unable to keep up with the pace of discovery of new proteins. It has, therefore, become imperative to develop methods, far more reliable than those currently available, for the prediction of structure from amino-acid sequence. The reliability of the prediction methods will improve substantially only after an intimate understanding of the physico-chemical interactions that dictate protein folding is obtained. (vi) Ligand binding, protein–protein interactions and other aspects of protein structure–function relationships will be understood at the molecular level only after the principles governing protein folding are first elucidated.

Conceptual models

The necessity for folding pathways

The unfolded polypeptide chain of a protein can adopt very many possible conformations. A polypeptide chain with $n+1$ residues, each with an average m equally probable conformations, can have m^n possible conformations. Thermodynamic analysis of protein unfolding[54] suggests that $m \approx 8$ in a real, unfolded polypeptide chain. For a small protein of 100 amino acid residues, this suggests the occurrence of 8^{99} or 10^{89} conformations. Even if each conformation were sampled for only 10^{-13} seconds, which corresponds to the fastest rate of interconversion, it would require more than 10^{66} years for the polypeptide chain to sample 10^{89} conformations[55]. Real proteins fold, however, within seconds or minutes. This discrepancy between such a calculation and experimental results is known as the 'Levinthal Paradox', and led Levinthal to propose that there must be pathways for protein folding, which speed up the process markedly[56,57]. More recently, it has, however, been shown that if instead of a completely unbiased random search, a small and rational energy bias against locally unfavourable conformations is included in the calculation of the time taken to fold, Levinthal's time is reduced to a biologically meaningful time[55].

Framework model

According to the framework model[58], protein folding is hierarchical: during folding, secondary structural elements are formed initially, which then constitute the framework for subsequent formation of tertiary structure. A more extended interpretation of this model is that when folding commences, fluctuating segments of secondary structure first form and then coalesce so that the polypeptide

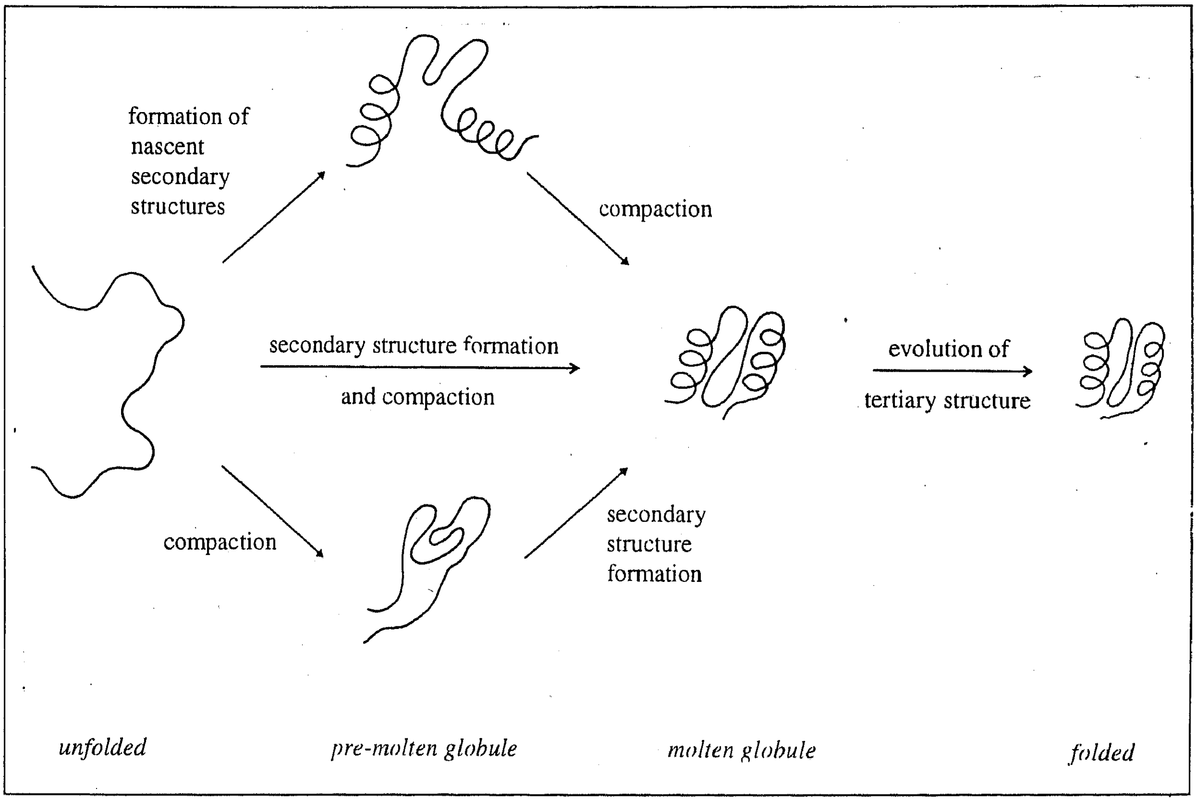

Figure 1. Models of protein folding. In the framework model (top pathway) nascent, fluctuating secondary structural elements form initially along the polypeptide chain. These coalesce to form a molten globule intermediate. In this model, the pre-molten globule is less compact, and any secondary structure present is very much more unstable than that in the molten globule. In the hydrophobic collapse model (bottom pathway), a non-specific collapse of the polypeptide chain occurs initially, and this facilitates subsequent formation of secondary structural elements, leading to the formation of the molten globule intermediate. In this model, the pre-molten globule is a disordered (structureless) form that is nearly as compact as the molten globule. The middle pathway depicts both collapse and secondary structure formation occurring simultaneously to yield the molten globule intermediate. In each case, the final tertiary structure evolves slowly from the molten globule intermediate.

chain becomes compact; the final tertiary structure forms subsequently. In part, this model reflects the realization that many proteins appear to have been assembled from segments of secondary structure[59,60]. It would appear that a consequence of the framework model would be that local conformation should depend on the local sequence. This appears, however, not to be true in native globular proteins: the same sequence can very often form different structures[61]. Nevertheless, experimental evidence for the framework model is strong[62–64]. It should be noted that elements of secondary structure (α helices and β-sheets) may not be stable sub-structures. This is, however, not an essential requirement for their assembly as long as once assembled, the complex of two or more secondary structural elements is more stable than the individual components. The diffusion–collision–adhesion model[65,66] proposed for the assembly of secondary structural units only demands that they be marginally stable and that the complex be more stable.

Hydrophobic collapse model

In the hydrophobic collapse model, the hydrophobic interaction, which can be described intuitively as water driving two non-polar molecules together, so that their contact with water is reduced, is thought to be the dominant interaction in determining the folding of a protein[67,68]. The idea is supported by the observation that the interiors of globular proteins comprise generally of hydrophobic amino acid residues. The non-specific nature of hydrophobic interactions suggested that they could form more quickly than other more specific interactions. This led to the idea of a hydrophobic collapse being the initial step in folding, in which non-polar side-chains are buried in a loose compact structure. Suggestive evidence for a rapid hydrophobic collapse has been found in staphylococcal nuclease, where C-terminal deletion mutant proteins are unfolded but compact and contain substantial secondary structure[69], and in kinetic folding experiments with dihydrofolate reductase (DHFR)[70]. The first direct evidence for the hydrophobic collapse model has been found in the case of barstar, where it has been shown that a non-specific hydrophobic collapse precedes the formation of any secondary structure or native tertiary interactions[71].

Nucleation–condensation–growth model

According to the nucleation–condensation–growth model[57,67,72–74], a region of the polypeptide chain serves as a nucleus for chain propagation to obtain the native state. The nucleus is expected to be unstable by itself, and is therefore present only fleetingly in the denatured state. Since the initial step is rate limiting, no intermediates beyond the nucleus are expected to accumulate in sufficiently detectable quantity, and the folding reaction is approximated by a two-state transition. The nucleation unit must be small enough for random search, and is expected to fully develop only in the transition state of folding. Direct evidence for the nucleation–condensation model is strongest for the folding of chymotrypsin inhibitor 2 (CI2) (ref. 74).

Jig-saw puzzle model

According to this model[75] of protein folding, the assembly of structural elements can occur in many different ways, analogous to the assembly of the pieces of a jig-saw puzzle which can be put together in various ways. There is no definite order to their assembly. In other words, there may not be any unique pathway for the folding of any protein. Recent studies suggest, however, that there are not a large number of equally accessible pathways by which a protein can fold; instead, folding occurs along one or a few well-defined sequential pathways with unique intermediates.

Development of structure during folding

The most useful way to analyse the pathway by which a protein folds is to characterize structurally and energetically all the intermediates that define the pathway[24]. Although new methodologies have been developed[62–64,76], partly folded intermediates that can be detected in kinetic studies are difficult to study because they have only a transient presence on the kinetic folding pathway. The cooperative nature of folding does not usually allow any intermediate to accumulate to any significant extent, and it is therefore difficult to characterize structurally a kinetic unfolding intermediate. Nevertheless, much information on how structure develops during folding has been obtained recently.

Early events in protein folding

The earliest steps in protein folding are usually studied using rapid kinetic measurements in the millisecond time domain. For many proteins, the earliest events of structure formation take place within the first 2–4 milliseconds, before they can be observed experimentally. The 'burst phase' change in the experimental observable used to monitor folding reports on the very early formation of structure. Information on what structure forms first can also be obtained by examining the structural features of the native protein which might fold faster than the global folding event. For example, a fragment of a protein which can assume native-like structure in isolation might form first during folding. Residual interactions and structures in the unfolded state may also play important roles in initial structure formation during folding[13,74,77–82]. Predictions have also been made on the early events in protein folding based on known native structures[83–86].

Formation of secondary structure. Two powerful probes used to study the formation of early secondary structure are far-UV circular dichroism (CD)-monitored stopped-flow kinetics[87,88] and pulsed hydrogen exchange in conjunction with NMR[19]. Folding of the peptide backbone can be monitored by measuring CD in the far-UV region; for example, formation of α-helices can be monitored at 222 nm. Initial formation of secondary structure has been observed for many proteins in far-UV CD-monitored kinetic experiments with millisecond time resolution[88–96]. In most of the stopped-flow CD studies reported so far, a significant amount of signal indicative of secondary structure was observed to have developed within the dead time of the measurement, much faster than the formation of the native structure[90–94]. In some cases, no further changes in ellipticity were found, suggesting that all the native secondary structure is formed in the earliest step[94,95]. In the case of tryptophan synthase, an overshoot of far-UV CD signal was, however, observed which was attributed to transient α-helical structures, which may not be native-like[94]. A similar overshoot was also observed in the case of hen lysozyme[90], which was attributed not to the formation of secondary structure, but to transient chain interactions, possibly associated with a disulphide bridge[91]. In some cases, several kinetic phases after the burst phase have been resolved[89,92], which are difficult to assign to the formation of specific structural features[89]. In the case of DHFR, several late phases have been resolved and assigned to the continued development of helices and rearrangement of the central β-sheet[92].

Whereas CD provides a useful index of secondary structure formation without defining the origin of specific features, pulsed hydrogen exchange labelling methods can detect the formation of specific secondary structure during folding[19,86]. The pulsed hydrogen exchange method, in which the ability of secondary structure to retard the exchange of peptide bond protons to exchange with solvent protons is measured, also allows the determination of the stabilities of the secondary structures formed at various times during the folding process. Early formation of native α-helix and β-sheet has been observed in cytochrome c (ref. 63), RNase A (ref. 62, 97), barnase[64], α-lactalbumin[98] and several other proteins[86]. A very early collapsed intermediate on the refolding pathway of the very fast folding form of unfolded RNase A has been shown to possess some marginally stable native-like secondary structural units[99].

Pulsed hydrogen exchange experiments may not, however, detect the initial formation of isolated elements of secondary structure. This has been shown by a comparison of hydrogen exchange data with that from far-UV CD in the cases of hen lysozyme[90] and cytochrome c (ref. 89). The structure formed in the initial stages of folding appeared to be too labile to afford significant protection from the relatively intense labelling pulse used in these experiments[31,99]. Formation of mutually stabilizing clusters of secondary structural elements is probably required to provide protection from exchange. This has been substantiated by studies of peptide fragments of regions of proteins which are protected from exchange initially in folding[100–102]. Thus, the combination of these techniques suggests the formation of abundant, but rapidly fluctuating, secondary structure in the earliest observable folding intermediates of hen lysozyme and cytochrome c.

Collapse and clustering of side chains. One of the major questions about very early events in protein folding is whether the initial formation of secondary structure precedes a global hydrophobic collapse of the polypeptide chain or whether the secondary structure is formed as a consequence of such a collapse[103]. This is a difficult question to answer because methods for the determination of a collapse of a polypeptide chain by direct measurements, for example, of the radius of gyration[104], are not readily coupled to rapid data acquisition systems.

Fluorescence is very useful in this context, because of its sensitivity to solvation, and both intrinsic and extrinsic probes have been used to investigate folding reactions. The fluorescence intensity of tryptophan residues is used typically to monitor folding in most kinetic studies because it is sensitive to environment, and its variation can be easily monitored[105,106]. In the case of a protein with a single tryptophan residue, the change in fluorescence can be interpreted in terms of the change in its particular environment[107,108]. Tryptophans have been engineered into various sites of lactate dehydrogenase to study the kinetics of folding of different parts of the structure[109]. A single tryptophan has been engineered into the interior of ubiquitin to monitor hydrophobic clustering[110,111]. In each of these cases, burst phase fluorescence changes within the dead time (5 milliseconds) of the measurement were observed, indicating that at least some side chain interactions involving the novel tryptophan residue had already formed on this time scale. The burst phase changes in intrinsic fluorescence intensity indicate that some degree of solvent exclusion, characteristic of the formation of at least a rudimentary hydrophobic core occurs within 5 milliseconds after commencement of folding.

In the folding pathway of DHFR, two tryptophan residues form rapidly a specific, native-like contact following an initial burst in secondary structure formation[92]. Fluorescence anisotropy decay measurements used to monitor the binding of a hydrophobic dye, 1-anilino 8-naphthalene sulphonic acid (ANS), during the folding of DHFR showed fluorescence anisotropy decay curves after 20 ms of refolding equivalent to those measured for the native protein[112], indicating that the molecule is similar in size to the native protein at this time point. In the case of hen lysozyme, which has six tryptophans, the fluorescence change within the first few milliseconds of refolding indicates the formation of hydrophobic clustering of at least a few tryptophan residues, although no significant hydrogen exchange protection is found within this time scale[113]. The earliest events in the refolding of lysozyme include both formation of substantial secondary structure, as detected by far-UV CD, and hydrophobic clustering, as indicated by change in fluorescence intensity[114].

Extrinsic probes, like ANS, serve as alternative probes to measure the hydrophobic collapse of a protein during folding[115]. The fluorescence of ANS is quenched in water and increases when it binds to clusters of hydrophobic residues in proteins. For many proteins, ANS fluorescence passes through a maximum during folding when partially folded intermediates accumulate transiently. For several proteins, including α-lactalbumin and β-lactamase, very little enhancement of ANS fluorescence during the first few milliseconds of folding was observed, suggesting that there was no extensive hydrophobic collapse in the initial intermediates[116]. In those experiments ANS was, however, present throughout the folding process, and it has been shown that the binding of ANS to intermediates affects the folding kinetics[117]. A new pulse labelling protocol, in which ANS was added after different times of refolding, has indeed shown that both α-lactalbumin and carbonic anhydrase undergo hydrophobic collapse very early during folding[117]. In the case of lysozyme, maximum ANS binding occurs in a burst phase within the dead time, when a substantial tryptophan intensity signal also develops. These data suggest that, in lysozyme, considerable hydrophobic collapse takes place during the initial step of folding, along with secondary structure formation[114]. Time-resolved fluorescence energy transfer measurements have been used to show that the initial folding intermediates of an immunoglobulin light chain[118], barstar[71] and apomyoglobin[119] are compact.

Direct measurements of protein compactness by small angle X-ray scattering have been performed recently both in equilibrium[120] and kinetic[121] experiments. By coupling this technique with rapid mixing, the compactness of an early intermediate in apomyoglobin could be determined in real time, within 100 milliseconds of commencement of folding[121]. Measurement of the scattering intensity spectrum indicates that the protein is indistinguishable in terms of its compactness from the native state much before folding is complete.

Side chain packing at the atomic level during folding has been studied recently by both one-dimensional[122,123] and two-dimensional[124] NMR in real time for systems that fold relatively slowly. Using this technique, the burst-phase intermediate of α-lactalbumin has been shown to resemble the acid-induced equilibrium intermediate of the same protein, which is a compact 'molten globule'[123].

The formation of molten globule intermediates is emerging as a common feature on the folding pathways of many proteins. Molten globules possess partial secondary structure stabilized by minimal native-like tertiary interactions, if any. The clustering of hydrophobic residues in these intermediates is monitored easily by their ability to bind hydrophobic dyes such as ANS[115,117,125]. Molten globules were first extensively characterized as equilibrium intermediates[38,116,126–128]. Recent studies have, however, shown that they do actually accumulate on the kinetic folding pathways, very often in a burst phase of a few milliseconds after commencement of folding[98,129]. While it is known that the equilibrium molten globules are compact, the degree of compactness in kinetic molten globules can only be inferred from their equilibrium counterparts.

Spectroscopic techniques that are available currently can measure the structural changes during folding only in the millisecond time scale, because the mixing deadtimes of the mechanical mixing devices are, with very few exceptions[130], typically 1–4 milliseconds. Thus, folding events occurring earlier than that are not observable. Methods are, however, being developed to probe structural events occurring on faster time scales by changing the physical[81,131–134] or chemical[135,136] conditions of the sample to initiate the folding reaction. Temperature jump experiments have been carried out in the case of cold-denatured barstar[81] and apomyoglobin[132] to study folding events in the microsecond timescale. Apomyoglobin forms a compact state within 20 microseconds, involving at least the A- and the H-helix backbones[132,133]. Submillisecond refolding events in the case of cytochrome c have been studied by initiating the refolding reaction either by laser-induced dissociation of a ligand[135] or by rapid photochemical electron transfer[136]. Analysis of the broadened NMR resonances, arising from the fast interconversion of folded and the unfolded species, has been applied to investigate submillisecond folding events in the case of monomeric repressor[137,138].

Kinetic barriers and fast folding reactions

The list of proteins that fold very rapidly (> 10 s^{-1} at the equilibrium unfolding transition midpoint at 25°C) is growing and includes cold shock protein B (CspB) (ref. 139), fragments of Streptococcal G protein[140,141] and cytochrome c (ref. 142). The rate of folding of a protein appears not to depend specifically on its size or structure, although in general, larger proteins fold more slowly. There also does not appear to be any correlation between stability and folding rate. Several marginally stable proteins, including CspB and a fragment of Streptococcal G protein[141], fold very rapidly. A mutation that decreases the stability of barstar also increases its rate of folding[143]. RNase A folds slower in conditions where it is more stable, and faster in conditions where it is less stable[144]. On the other hand, a relationship between the rate constant of folding and the stability of the native conformation has been demonstrated in the case of the Arc repressor[145] and ubiquitin[111].

The reasons why folding rates of proteins of similar size vary over a 100-fold range are not understood. Tertiary interactions of native[146] or non-native[142,147] origin sometimes act as kinetic barriers in folding. In the case of cytochrome c, a non-native interaction between the unfolded protein and the heme group is responsible for the slow folding of the protein; elimination of the interaction causes folding to occur 20-fold faster[142]. In the refolding of dimeric Arc repressor, formation of a set of native, partially buried, salt-bridge and hydrogen-bond interactions acts as a kinetic barrier. Replacement of these specific interactions with non-specific hydrophobic interactions accelerates the refolding rate of the protein by three orders of magnitude[146]. Equilibrium molten globules have a tendency to form soluble aggregates in solution, a property likely to be shared by kinetic molten globule intermediates. Disaggregation of a transiently formed kinetic molten globule aggregate on the folding pathway might also act as a rate limiting step during folding.

The transition state in protein folding

The transition state in a protein folding reaction is the structure that is at the highest energy level on the pathway of folding. The classical transition state theory of chemical reactions, proposed by Eyring[148], is applicable to chemical reactions involving the making or breaking of covalent bonds. In contrast, the protein folding reaction involves formation or destruction of mainly weak interactions. Nevertheless, transition state theory appears to be applicable to folding reactions as well. The results of kinetic studies for a few proteins have indicated that the transition state is compact[149] and more native-like than unfolded[150].

Structural information on the transition state of folding can also be extrapolated from characterization of the intermediates that precede the transition state on the folding or the unfolding pathway of a protein. Unfolding and refolding are linked by the principle of microscopic reversibility, requiring that under the same conditions, transition states and intermediates for unfolding and refolding should be identical[151]. In the case of the folding of RNase A in the absence of complications due to non-native interactions, it has, however, been proposed that the transition states on the folding and unfolding pathways are not identical[144].

The advantage of analysing the unfolding pathway is that it starts from the best defined structure on the folding pathway, that of the folded state. In addition, there are no complications arising from slow phases due to proline isomerization. The unfolding kinetics of most proteins have been described by simple two-state transitions, without the involvement of any unfolding intermediates. Recent studies on RNase A by NMR[152], and on barstar by optical probes[153] have, however, demonstrated the accumulation of kinetic intermediates on the unfolding pathway. The kinetic unfolding intermediate of barstar is compact with a dehydrated hydrophobic core and devoid of substantial secondary structure[153]. The unfolding of a low-pH equilibrium intermediate of a mutant form of apomyoglobin shows biphasic kinetics with a large burst phase change in fluorescence[154]. This has, however, been explained in terms of the intermediate being an ensemble of structurally related forms.

The transition state for the folding reaction, in which many hundreds of non-covalent interactions form, may not be unique, but may represent an ensemble of states with very similar energies, or one of several alternative states that are available. Theoretical studies suggest the latter possibility[155], but the demonstration that several proteins show apparent two-state folding/unfolding kinetics[139,156,157] suggests that if multiple transition states are present, they are equivalent in energy.

Late events in protein folding

The folding kinetics of most proteins, with few exceptions[139,157,158], show at least two distinct kinetic phases, usually a fast phase and at least one slow phase, with their time constants significantly different, even when equilibrium studies indicate a simple two-state unfolding transition. As discussed above, major structural changes towards the folded state like the hydrophobic collapse, H-bond formation, the packing of secondary structural elements, etc., appear to take place within the time scale of the fast phase. For many proteins, the reason for the slow processes is the isomerization about proline imide bonds[159–161]. It has been assumed generally that little structure formation accompanies this isomerization process, and for this reason, slow folding reactions have been used as model systems for the quantitative analysis of the coupling of intrinsically slower chemical processes (e.g. imide isomerization) to faster structure-forming folding reactions[162–164]. The extent of structure formation that occurs in a slow folding reaction has, however, not been well studied for any protein. In particular, the consolidation of tertiary interactions that must occur in this step is poorly understood.

The *X-Pro* imide bond, where *X* is any amino acid residue, is found to be in a *cis* conformation with reasonable frequency (approximately 7% of all prolyl peptide bonds) in many native proteins, whereas *cis* amide bonds have been observed only rarely[165]. In unfolded proteins and in peptides, the *trans* form of the *X-Pro* bond is favoured thermodynamically over its *cis* isomer, and the equilibrium distribution of the two forms are 70–90% and 10–30% for *trans* and *cis*, respectively[166,167]. The presence of these two forms in unfolded proteins gives rise to heterogeneity of the unfolded form[82]. The rates of *cis* ⇔ *trans* interconversions are slow and in the same time range as slow refolding reactions. This led Brandts and his colleagues[168] to propose that the slow phase of protein folding is because of the slow isomerization step from the non-native *trans* form of proline to its native *cis* form during folding. Many model proteins used for folding studies, such as RNase A, RNase T1, thioredoxin and barstar, have *cis* imide linkages, whereas others, such as hen lysozyme, T4 lysozyme, cytochrome c, etc., have only *trans* prolines. The refolding of proteins with one or more native *cis* prolyl imide bonds is usually dominated by slow kinetic phases, while the refolding of proteins containing all *trans* prolyl imide bonds is dominated by fast phases.

Proline isomerization has been implicated directly for being responsible for the slow refolding kinetics of RNase A (refs. 160,169,170), RNase T1 (ref. 159), staphylococcus nuclease[161] and thioredoxin[171]; replacement of *cis* prolines leads to the elimination of the slow-refolding reactions in these proteins. This effect is, however, not seen in the case of human lysozyme[172], indicating that factors other than *cis-trans* isomerization of *X-Pro* bond could also be responsible for slow-folding reactions in some proteins. A cystine knot loop threading mechanism has been suggested to be responsible for the slow phase in the unfolding of human beta-NGF[173].

Two slow phases are found in the refolding of two-disulphide derivatives of bovine pancreatic trypsin inhibitor (BPTI). Using energy calculations, Levitt[174] predicted that the native state was destabilized most strongly in molecules with *Pro8* in the non-native *cis* conformation (all prolyl peptidyl bonds are *trans* in native BPTI). This prediction was later confirmed experimentally[175]; replacing *Pro8* by glutamine in a two-disulphide variant of BPTI abolished the slowest phase of folding. This result implies that the incorrect conformation of a proline residue that destabilizes the native conformation the most, also leads to the strongest deceleration of folding.

Structural characterization of folding pathways by protein engineering methods

The advent of recombinant DNA techniques, which led to the birth of protein engineering, provided a powerful tool to study the contributions of individual amino acid residues to the conformation and folding of a protein. One of the principal goals of protein engineering methods has been to estimate the informational content of individual amino acid residue positions. The challenge is to determine the contributions of individual residues to the stability and folding of a specific protein. By careful equilibrium and kinetic analysis of mutant proteins, invaluable information can be obtained on the folding pathway of a protein, which is otherwise not available from the studies on the wild type protein.

Protein engineering and equilibrium intermediates

Folding intermediates are populated usually to a negligible extent at equilibrium because they are less stable than the fully folded protein. Thus, many of the small monomeric, single-domain proteins unfold through a highly cooperative process with no detectable accumulation of equilibrium intermediates between the fully folded and the unfolded forms[32]. Site-directed mutagenesis is a potential method for breaking down the cooperativity of the folding process so that folding intermediates accumulate at equilibrium and are more amenable to study[176]. Equilibrium folding intermediates have been populated to a significant extent by perturbing hydrophobic interactions in barnase[177] and by perturbing a buried hydrogen bond in barstar[178] and their structures have been studied spectroscopically.

Double mutant cycles

The extent of interaction between two residues in a folded protein can be estimated by using double mutant cycles[179,180]. The method consists of identifying two interacting residues in the folded protein and mutating them both independently and simultaneously. Then the differences in free energy of unfolding ($\Delta\Delta$Gs) of the three mutant proteins are measured with respect to the wild type protein. The comparison of the difference of the $\Delta\Delta$Gs of the two single mutant proteins with that of the $\Delta\Delta G$ of the double mutant protein indicates the extent of interaction between the two residues under study. The double mutant cycle allows the measurement of the changes in cooperativity along the folding pathway. This approach has been applied to barnase and the results indicated that during the refolding process cooperativity is still present in some regions of the folding intermediate[181].

Characterizing the transition state by protein engineering methods

Protein engineering methods offer a glimpse of the structure of the transition state of folding. Structural characterization of the transition state is achieved from the inferences of kinetic measurements on mutant proteins[24,64,151,182–184]. Sufficient experimental progress has now been made to validate this method and the theoretical basis is reasonably well established[185].

The method comprises of: (i) establishing and measuring the interactions that stabilize different parts of the molecule from experiments on the equilibrium stability of mutant proteins and (ii) using kinetic measurements of protein folding and unfolding to determine what fraction of the stabilization energy measured in (i) is used to stabilize the transition state for folding[182]. Each mutation engineered into the protein acts as a reporter group for the progress of folding at the appropriate position. The procedure gives a quantitative parameter, Φ, for the localized comparison of the transition state with the native state[182]. Φ is calculated as the change in the free energy difference between the transition state and the unfolded state relative to the change in the overall protein stability as a result of the mutation. In situations where a mutation has an identical energetic effect on the folded and transition states, $\Phi = 1$ and the interaction is assumed to be present in the transition state. In cases where $\Phi = 0$, the structure of the transition state in the region is assumed to be identical to that of the unfolded state. Fractional values of Φ, found frequently in the analysis of measurements of the CI2 folding, signify that the structure is weakened or that a dynamic equilibrium exists between folded and unfolded conformations. These fractional values of Φ are particularly difficult to interpret in structural terms[73,186].

Using the above method, the structures of transition states of several proteins have been characterized. The transition state of CI2 appears to be a globally collapsed state strongly resembling the folded protein but expanded by 30%, and lacking the specific hydrophobic packing characteristic of the native state[186–188]. Structural characterization of the transition state of barnase by protein engineering reveals that it is very similar to the folded state[23,182,184]. The major differences are a weakened main hydrophobic core, the disruption of a small subdomain and of some loops, and the unwinding of the amino termini of the two helices, and less secondary structure. As in the case of CI2 and barnase, considerable secondary structure is formed only after the transition state in folding of Arc repressor[189], CheY (ref. 190) and a fragment from GCN4 (ref. 191). Molecular dynamics simulation studies have also been used to study the structures of the transition states of CI2 (refs 192,193) and barnase[194]. A good correlation between the experimental and the theoretical results[195] corroborates the validity of the procedure of studying transition states by protein engineering.

Characterizing kinetic folding intermediates by protein engineering

Although spectroscopic probes such as fluorescence and circular dichroism provide useful information about structure, both at equilibrium and in kinetic studies, they monitor the structure at a macroscopic level. The structural information at the level of individual residues and atoms at equilibrium can be obtained only by NMR. The study of kinetics of structural changes at the atomic level during folding by stopped-flow NMR spectroscopy, which could be the best method to monitor protein folding, is, however, still at an early stage[123–124,152,196].

There are two existing procedures for analysing the structure of the transient intermediates with high structural resolution: pulsed hydrogen exchange[19,86,197] and protein engineering[198]. Although the protein engineering method reports only indirectly on structure, it is more powerful in being applicable to many more regions of the protein, including all the side chains and the hydrophobic core, and it is not just restricted to those NH groups that are protected sufficiently well to be resistant to exchange. Further, the protein engineering method gives information on energetics, and may also be extended to events that occur on shorter time scales than pulsed hydrogen exchange methods[73,81].

Protein engineering methods have been used to characterize transient intermediates on the folding pathways of barnase[198,199] and phosphoglycerate kinase[200]. The transient folding intermediate of barnase is characterized by relative positions of α-helices and β-sheet being native like, as well as the relative positions of β-strands within the sheet being native-like. The structure looks very much like the transition state, except that the intermediate has a weaker hydrophobic core[198,199]. The structure of the transient intermediate determined from protein engineering studies agrees well with that obtained from hydrogen exchange studies.

Engineering disulphide bonds to study protein folding

Protein folding has been studied by kinetic analysis of disulphide bond formation in proteins like BPTI, which requires formation of specific disulphide bonds for its folding[201]. Folding pathways of such proteins have been studied and trapped intermediates have been characterized by selectively mutating cysteine residues and measuring the rate of disulphide bond formation[202]. Engineered disulphide bonds have been used to report on the folding of specific regions of a protein[203], where formation of a disulphide bond is not required for folding. The engineered disulphide bond is expected to stabilize an early kinetic intermediate if the bond is located in the region which folds early in the pathway.

The introduction of free cysteine residues in phosphoglycerate kinase and the analysis of their reactivities during the folding reaction has also provided useful structural information about kinetic intermediates[204]. These studies suggest that the kinetic folding intermediates contain an ordered native-like secondary structure, especially near the centre of the protein, that is weakened at the periphery.

Concluding remarks

Experimental studies of protein folding are being provided with valuable insight from simplified statistical models[36,37,42]. Theoretical studies have now become sufficiently sophisticated to be able to describe many of the basic features of folding that are evident from experimental studies[155,192–195,205–208]. Clearly, the study of protein folding will benefit tremendously from the marriage of theoretical and experimental studies.

The results of experimental studies with millisecond time resolution on the folding pathways of many proteins, including RNase A (ref. 62,99), α-lactalbumin[98], lysozyme[30], apomyoglobin[129], cytochrome c (refs 89,135) and DHFR[95,112] suggest that compaction of the polypeptide chain and secondary structure formation occur simultaneously, because these two processes cannot be resolved temporally. It remains to be seen, whether the two processes indeed occur simultaneously in a faster time domain. Folding experiments have only recently entered the microsecond and nanosecond time domain, and the possibility of nanosecond circular dichroism measurements[209] holds great promise. Future studies will provide crucial information on how folding commences. In the case of barstar, it has been shown that a hydrophobic collapse precedes secondary structure formation[71], leading to the formation of a disordered (structure less) globule, and recent temperature jump experiments in the microsecond time scale[81] appear to support this result.

Partially folded forms of a protein can often be detected in equilibrium with the fully folded form, and much recent effort has gone into obtaining structural information on these forms[39], and relating them to structural intermediates that accumulate transiently on the direct folding pathway. In particular, hydrogen exchange measurements of proteins in the presence of mildly destabilizing concentrations of denaturants[210–214] yield structural information on partly unfolded forms of the protein in rapid equilibrium with the fully folded protein. These partly folded forms may or may not, however, represent structural intermediates on the direct folding pathway[213]. While in the case of cytochrome c, the partly unfolded forms do resemble structural intermediates that have been identified on the kinetic folding pathway[211], in the case of barnase, the partly unfolded form does not resemble the kinetic intermediate that has been structurally characterized[213]. It is essential to determine if partly folded forms of a protein, in equilibrium with either the unfolded state (in kinetic experiments) or with the folded state (in hydrogen exchange experiments), are tangibly present on the direct folding pathway. It has been shown recently that a non-native intermediate accumulates during the folding of β-lactoglobulin[215]. This has again highlighted the importance of demonstrating whether partly folded intermediates, which are observed in kinetic experiments, accumulate on the direct folding pathway or whether they are off-pathway dead-end intermediates[20].

Although it has now been clearly established that many proteins fold along one or more well-defined pathways, which involve intermediate structures that are only partly folded[16,27], recent studies have shown that the folding/unfolding of small proteins, such as CspB (ref. 139), CI2 (ref. 156), the IgG binding domain of streptococcal protein G (ref. 141), acyl-coenzyme binding protein[156], the SH3 domain of spectrin[217] and cytochrome c (refs. 142, 216) follow two-state kinetics: no intermediates are detectable between the unfolded and the folded state[218]. These observations have raised the question whether intermediates are essential because they guide the folding process along a pathway and thereby speed up the folding process, or whether they are only incidental to the folding process and accumulate simply because of the presence of kinetic barriers. Do intermediates that are too unstable to be optically detectable exist on the folding pathways of proteins that fold by apparent two-state behavior, where they function in a spectroscopically silent manner? Does the accumulation of a stable intermediate on a folding pathway actually cause folding to proceed slower than it would in its absence[64,111,120,144,219]? Identification of the natures of kinetic barriers that retard folding, and their structural description are major goals in the study of protein folding.

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Evaluation of a light cured dental composite containing a radiopaque glass filler

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Light curing dental composite pastes were prepared using an indigenously developed Bisphenol A-glycidyl methacrylate (BIS-GMA) resin and a commercially available radiopaque glass filler of different particle sizes. SEM examination of the virgin filler and composite surface revealed agglomeration of particles in virgin state and fine encapsulation of the filler particles in resin matrix of composite. Variation of the filler content shows that an amount of 220 phr (parts per hundred of resin mixture) of the radiopaque glass filler of larger grain size or 250 phr of the smaller grain size incorporated into the paste tends to improve the compressive strength and diametral tensile strength considerably compared to composite containing conventional quartz fillers.

DENTAL composites generally consist of a dimethacrylate organic binder resin such as bisphenol A-glycidyl methacrylate (BIS-GMA) and an inorganic resin filler such as quartz or glass modified with a silane to effect a strong bonding between the resin and the filler incorporated into the resin matrix. The nature and size of the filler play a dominant role in determining the final properties of the composite and its performance in the oral cavity[1]. Filler particles are found to have a particle size < 50 μm in conventional composites whereas microfilled composites contain fillers around 400 nm. Glass fillers with barium or strontium incorporated into it to induce radiopacity is widely used in many composites[2]. These radiopaque composites are widely used in posterior applications where stress-bearing materials are required and easy access to the clinician for examination is denied.

The development of the BIS-GMA resin monomer indigenously has been reported earlier[3]. Both chemical curing and light curing dental composites using this monomer resin and quartz as the filler were developed subsequently and subjected to exhaustive studies[4–7]. However, the need for radiopacity for monitoring the restored teeth prompted us to use a new radiopaque glass as filler material and the effect of the amount and particle size of this filler upon the properties of the light curing composites developed is reported here.

BIS-GMA resin synthesized in the laboratory and a radiopaque glass filler (GM 27884, M/s. Schott Glasswerke, Landshut, Germany) was used for the study. Three different grain sizes of the same glass were used [IDENT-NR-642907304 (0.7 μm), IDENT-NR-642907604 (1 μm), IDENT-NR-642907407 (1.5 μm)]. The composition of the glass is reported as SiO_2 55, BaO 25, B_2O_3 10 and Al_2O_3 10. The particle size distribution of the glass powder was determined using a particle size analyser (Galai Cis, Israel). A crosslinker triethyleneglycol dimethacrylate was used to thin down the viscous BIS-GMA to form the resin mixture and a photoinitiator (–) camphorquinone (both from Aldrich Chemical Co., USA) and other stabilizers were incorporated during paste preparation.

The glass filler particle was silanated using a 1% 3-(trimethoxysilyl) propyl methacrylate (97% Aldrich Chemical Co., USA) solution in acetone to ensure strong adhesion between the filler and the BIS-GMA resin. The solvent was allowed to evaporate at 40°C and the coated filler was subsequently heated at 120°C for 60 min and cooled in airtight containers. The silanated filler was added to the resin mixture in different amounts of 205, 220, 250 and 280 phr. They were mixed well in an agate mortar and pestle till a fine dough-like consistency was achieved. Separate pastes were prepared using particles with different grain sizes.

Compressive strength (CS), diametral tensile strength (DTS) and microhardness (MH) were determined for dental composites prepared by light curing the composite pastes. Specimens of size 3 mm diameter and 6 mm height were made for CS measurements by curing the composite pastes in a brass mould kept between two glass plates and held using a C-clamp. The samples were exposed to a visible light source (440–480 nm wavelength, Caulk the Max, UK) for 60 s each on both sides for curing. Subsequently the samples were released from the mould and the inner surfaces of the cured composite were exposed to the light source for a further 20 s to ensure uniform curing within the core of the material. At least ten specimens of each paste were thus cured and stored at 37°C for 24 h before testing.

DTS specimens of size 6 mm diameter and 3 mm height were prepared similarly using a stainless steel mould. Paste packed in the mould between glass slides were exposed to the same light source as above for 60 s each on both sides for curing. The cured specimens were taken out and stored at 37°C in distilled water for 24 h before testing.

Compressive and diametral tensile strength values were evaluated using a universal testing machine (INSTRON Model 1011, UK) using a reported procedure[8]. The crosshead speed of 10 mm/min was maintained for all testings. At least 6 testings were carried out for each sample and mean and standard deviations calculated. For DTS measurements a minimum of 10 specimens were tested. The load at which break occurred was noted and the CS and DTS values were calculated using the equations

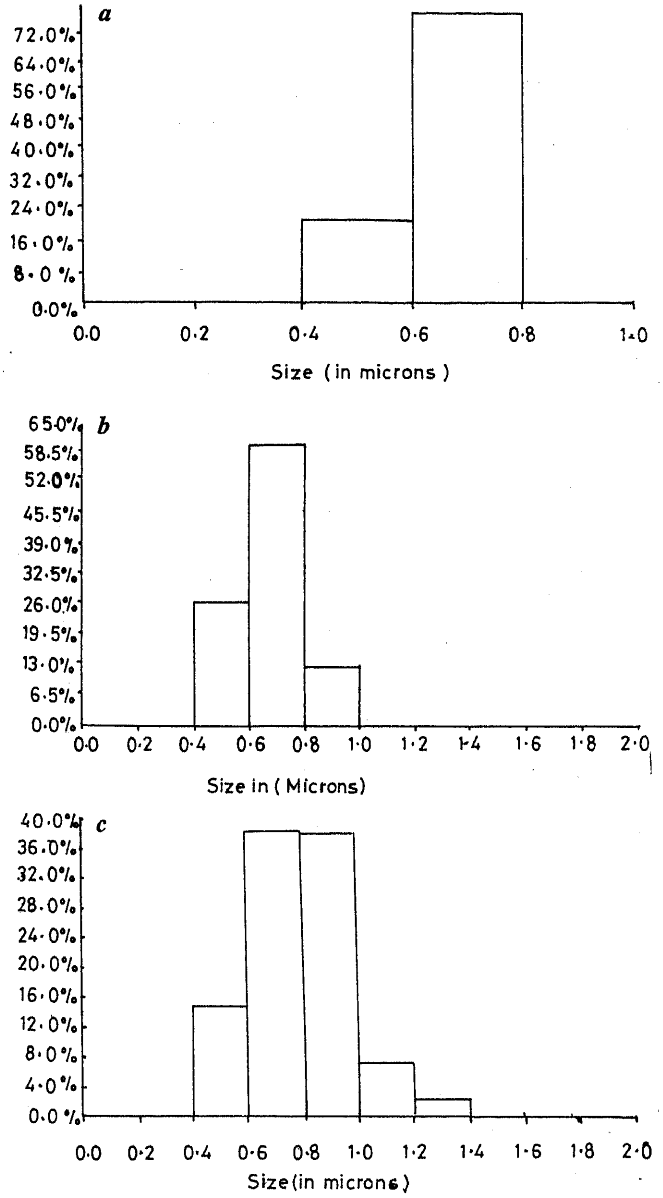

Figure 1. Particle size distribution profiles for glass fillers of grain size 0.7 µm (*a*), 1.0 µm (*b*) and 1.5 µm (*c*).

$$\text{CS (in MPa)} = P/\Pi r^2,$$

where P is the load applied in newtons, r is the radius of the specimen in mm.

$$\text{DTS (in MPa)} = 2P/\pi DL,$$

where D is the diameter of specimen in mm and L is the height of the specimen in mm.

Specimens for surface microhardness were prepared as was done for DTS using the same mould. For each testing, two specimens were used. The surface microhardness was measured on each side using a Vickers microhardness tester (Carl-Zeiss, Jena). At least 8 measurements for each specimen were done and average taken. The diamond indentor fitted is a square-based pyramid suitable for Vickers hardness measurements. The specimen was placed flat on a glass slide and mounted on a holder on the microscope stage. The specimen surface was examined microscopically and the indentor was then moved into position and the microscope stage was raised steadily until the required load was applied by the indentor upon the specimen. In all cases, a load of 100 g was used. The load was held for 15 s before the microscope stage was steadily lowered. The indentor was then replaced with the objective lens and the image of the indentation was focused. The contrast of the image was optimized using differential filtering and the size of the diagonal of the indentation was measured. The Vickers microhardness (H_v) was calculated using the following equation

$$H_v \text{ (in kg/mm}^2) = 1854.4\, P/d^2,$$

where P is the load applied in gram, d is the length of diagonal in microns.

The surfaces of the composites were polished using silicon carbide papers of 240, 400, 600 and 1200 grit, in that order, and finally with 1 µm alumina powder slurry in water, coated with a layer of gold using a vacuum sputtering machine (E-101, Hitachi ion sputter, Japan) and studied using a scanning electron microscope (Hitachi Model S-2400, Japan, 1500–2000 ×).

Particle size analysis of the three glass powders revealed that the grain sizes ranged from 0.7 µm to 1.5 µm (Figures 1 *a–c*). It was found easy to incorporate the filler up to a level of 250 phr into the resin mixture. However, incorporating 280 phr of the filler into the resin was found very difficult especially in case of particles with grain size 1.5 µm resulting in pastes of extremely high viscosity. As a result, the mechanical properties could not be evaluated in this case. Similarly it was observed that the paste formed through mixing 205 phr of the filler was of very low viscosity and it was found difficult to cure samples with the light source due to the flowing characteristics of the paste.

Scanning electron micrographs (Figures 2 *a,b*) show the virgin and composite surface containing glass filler particles. While the virgin filler appears to be agglomerated due to its small size, the filler particles, after silanation, are found encompassed by the resin matrix, which ensures strong bonding at the resin/filler interface. Uniform distribution of filler particles could be observed at the polished composite surface.

Figure 3 shows the compressive strength values for the composite samples prepared using the radiopaque glass filler at different proportions of filler. The recommended values by various standards[9] and in literature[10] for properties of dental composites are given in Table

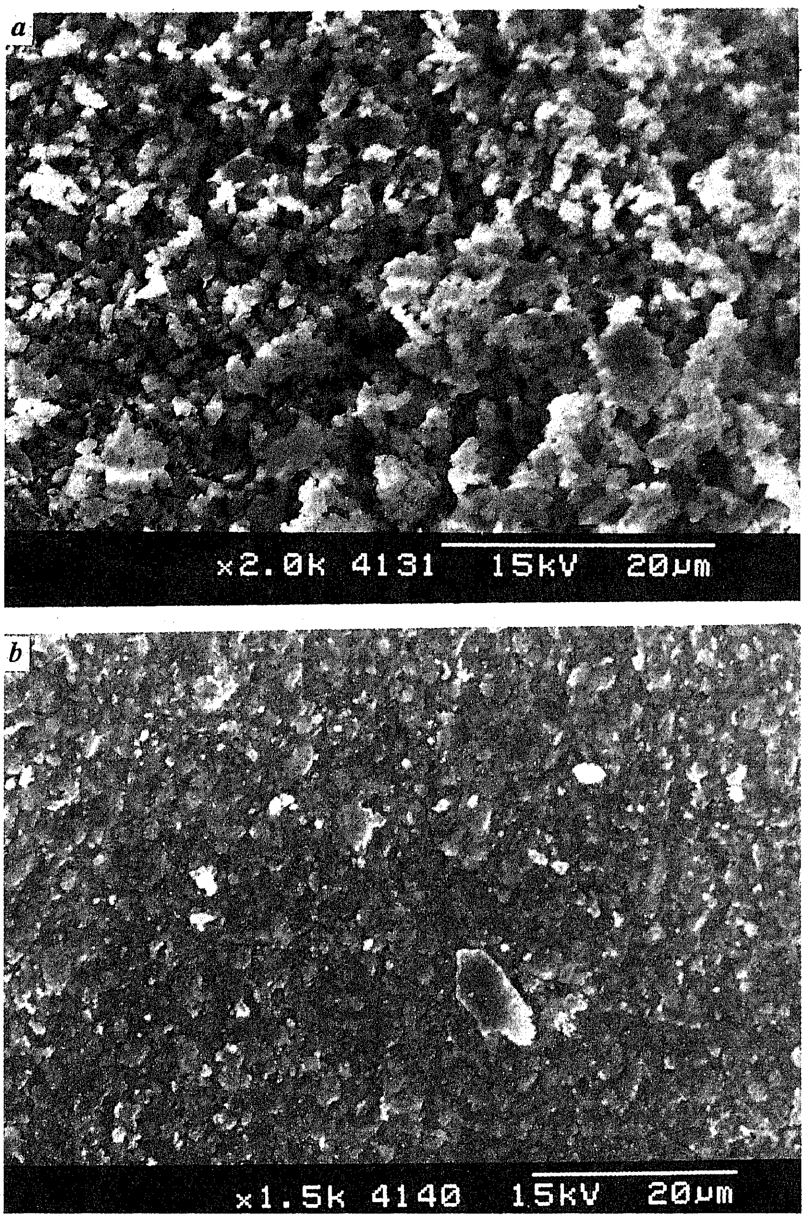

Figure 2. SEM picture of (*a*) virgin filler in agglomerated condition (× 2000) and (*b*) dental composite surface (× 1500) showing uniformly distributed fillers encapsulated with the resin matrix.

Table 1. Comparison of values for properties studied in case of light curing dental composites

Property studied	Recommended value[9,10]	Values obtained using conventional fillers[5]
Compressive strength (MPa)	> 220	220 ± 6*
Diametral tensile strength (MPa)	> 34	36 ± 2
Microhardness (kg/mm^2)	> 34	48 ± 10

*Standard deviation.

1. It can be clearly seen that an amount of 220 phr is found to give excellent CS values in case of particles at all three grain sizes, values reaching up to 300 MPa in certain cases. Higher concentration of filler up to 250 phr though favours composites containing small particles does not seem to agree for particles above 1 µm grain size as is evidenced in the deterioration of the value.

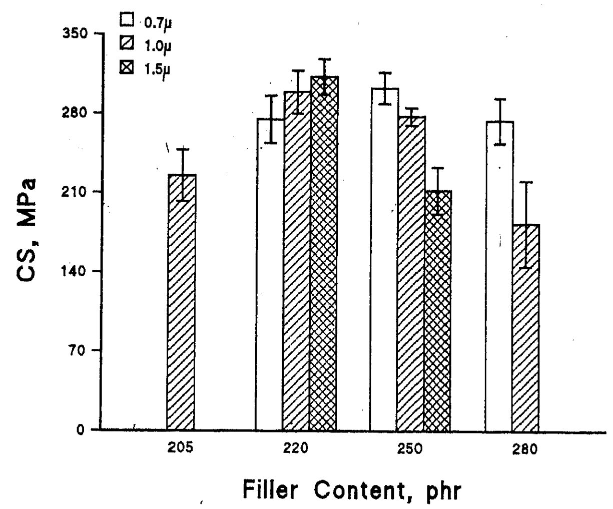

Figure 3. Bar graph showing compressive strength values for composites incorporated with 205, 220, 250 and 280 part per hundred of resin mixture of silanated glass filler GM27884.

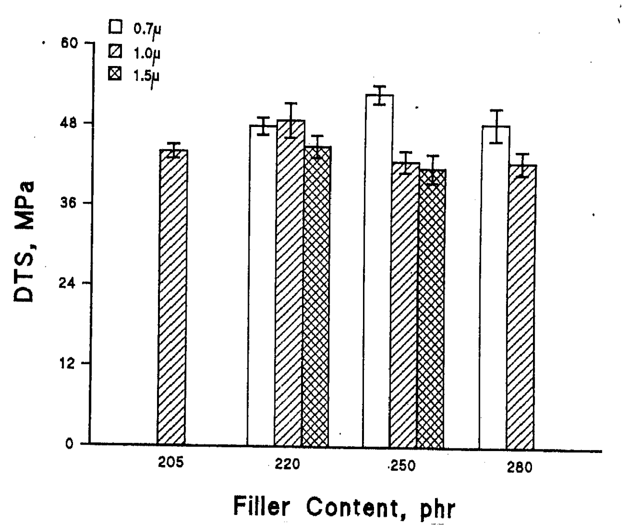

Figure 4. Bar graph showing diametral tensile strength values for composites incorporated with 205, 220, 250 and 280 part per hundred of resin mixture of silanated glass filler GM27884.

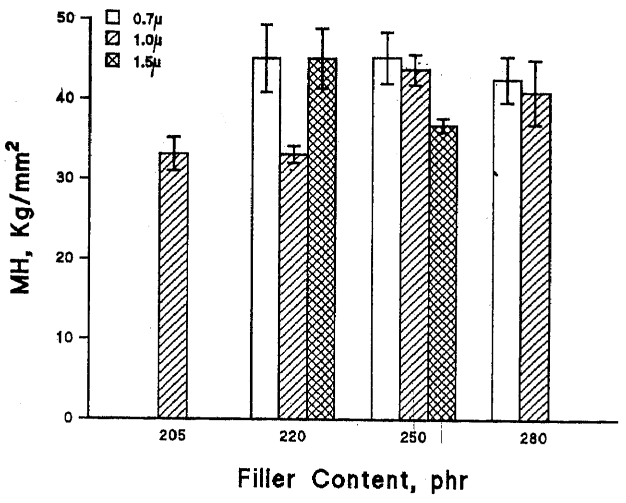

Figure 5. Bar graph showing microhardness values for composites incorporated with 205, 220, 250 and 280 part per hundred of resin mixture of silanated glass filler GM27884.

Observations of DTS values (Figure 4) also showed the same trend for the composite specimens. Dental composites containing 220 phr of the glass filler tended to show excellent strength values in the range of 45–50 MPa whereas the minimum stipulated value is about 34 MPa. As in the case of compressive strength, DTS was maximum for composites containing 250 phr of 0.7 μm grain size particles though with larger particles, properties tended to deteriorate. Microhardness values also tended to show high values (> 45 kg/mm^2) at filler content levels of 220–250 phr.

Finally, incorporation of radiopaque glass filler, especially at 220–250 phr range into the composite is found to improve the above properties when compared to the values obtained for composites containing conventional fillers such as quartz and silica[5] (Table 1). While compressive strength and tensile strength values are found to appreciate, the microhardness values are found to show comparable results. This can be attributed mainly due to the smaller and uniform particle sizes of the glass filler used (0.7–1.0 μm) compared to the larger and non-uniform size of the conventional quartz fillers (1–50 μm) used in the earlier composite.

In conclusion, about 220–250 phr of the radiopaque glass filler GM27884, varying between grain sizes 0.7 and 1.0 μm, when incorporated into the BIS-GMA resin matrix is found to impart better mechanical properties to a light cured dental composite compared to composites containing conventional quartz fillers.

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Transpiration in two common tree species of Auroville, Tamil Nadu, India, using the calibrated heat pulse method to measure sap flux

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The community of Auroville since 1968 has been reforesting large tracts of land on the Coromandel Coast near Pondicherry. Well over three hundred species of indigenous and exotic trees have been introduced to the area, as well as shrubs, grasses, and creepers. The groundwater has, however, witnessed a steep decline, yet rainfall patterns have not varied out of the ordinary. Although one of the major reasons for this depletion is the huge increase in borewell-irrigated agriculture, it was posited that the reforestation in Auroville must have played some part in modifying the water balance of the area. The purpose of this research therefore, is to understand in more depth, the role of Auroville's forests on the replenishment of groundwater, with a possible view to managing the forests of Auroville according to the requirements of the area's water balance. The technique employed here to determine transpiration is known as 'the calibrated heat pulse method'.

Measurements started in Auroville in March 1994, on two common tree species, *Acacia auriculiformis* and *Peltophorum pterocarpum*. The trees examined were planted in 1982, and the data, taken for five months between March and July 1994, showed that *P. pterocarpum* transpired more than three times as much as *A. auriculiformis*. Although the results were revealing, it was clearly seen that measurements would be required over a period of at least one year, and on more species of trees, to obtain a detailed hydrological picture of the area.

MEASURING the quantity of water transpired by trees has historically been difficult, cumbersome or both, with questionable data to finish with. Such methods have involved weighing the tree and its soil in a weighing lysimeter and measuring the resulting decrease in weight as the tree transpired or, alternatively, injecting dyes, solubles or radioactive materials to be 'traced', thereby giving the velocity of the sap in the tree. The latter methods in particular, physically harmed the tree and even destroyed it, making it alter its transpiration[1], otherwise, as with the lysimeter, trees were taken out of their natural context and estranged from the complex variables that nature brings into play.

The development of the calibrated heat pulse method overcame some of these problems. It involved minimal physical damage to the tree, and the equipment being light and easy to install, could be used in field situations.

Tracing heat pulses to determine sap velocity was developed by Marshall[1]. He envisaged a two dimensional heat flow in a medium of thermal homogeneity and uniform liquid flux – not likely in a tree, but close enough to reality to make the data valid. Marshall also discovered that the speed of the sap was actually greater than that of the heat pulse and put this down to the disturbance caused by the insertion of thermistors and heater into the tree. Most importantly, what Marshall noted was the constant relationship between the sap velocity and the heat velocity, allowing the difference to be quantified and calculated. Cohen *et al.*[2], who developed the present method using advanced electronic equipment, confirmed this in an experiment on citrus trees, kept under drought and well-watered conditions, in both cloudy and sunny weather. Results showed that the heat pulse method under-estimated transpiration by a mean value of 55.3% with only a 2.4% coefficient of variation.

Research using the calibrated heat pulse method is currently underway in Auroville, Tamil Nadu, India. The project was started after a seminar led to a discussion over the net effect of trees on the hydrology of the area, especially on the level of the water table which was declining steadily despite the extensive watershed work. Auroville, situated on a plateau, now consists of about 2,500 acres of land, approximately half of which has been reforested since the late 1960s.

Due to experimental constraints, only two tree species were measured with four replicates for each species. *A. auriculiformis*, the most dominant tree in the area, is a very fast growing and relatively hardy and drought-resistant exotic from Australia. The other tree selected was *P. pterocarpum*, a popular tree used primarily as an ornamental and alleyway tree.

In the calibrated heat pulse method, the heat wave velocity is measured after a half second heat pulse has been radially injected as a line source into the tree. The heat is transported by convection with the sap and measurements of the heat wave velocity are made by special microbead thermistors exactly 15 mm downstream from the linear heat source (that is 15 mm higher up the tree (Figure 1)). Each thermistor is capable of measuring at 1 cm increments into the tree for a total of 6 cm (Figure 2). Programme control and measurement of thermistor signals are made by a datalogger (Campbell 21X) through the intermediary of a custom-built thermistor and heat pulse junction box (Ariel).

As the heat wave passes the thermistors, the time to maximum temperature ($t_{\max}$) is recorded by the datalogger, giving the velocity of the heat between the linear heat source and the thermistor. The $t_{\max}$ values along with the areas of the presumed concentric rings of similar sap flux in the tree (Figure 2) are used in the formula modified by Cohen *et al.*[2] to give volumetric sap flux (for formula see Appendix 1).

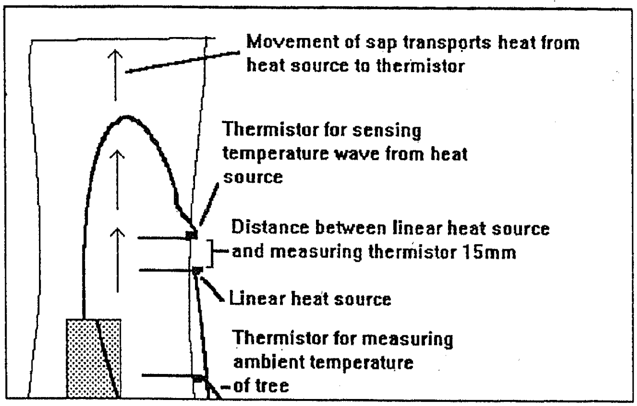
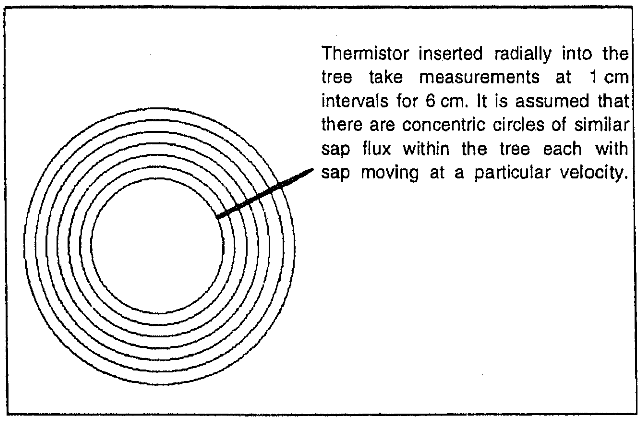

Movement of sap transports heat from heat source to thermistor
Thermistor for sensing temperature wave from heat source
Distance between linear heat source and measuring thermistor 15mm
Linear heat source
Thermistor for measuring ambient temperature of tree

Figure 1. Positions of heater and thermistors in relation to sap stream.



Thermistor inserted radially into the tree take measurements at 1 cm intervals for 6 cm. It is assumed that there are concentric circles of similar sap flux within the tree each with sap moving at a particular velocity.

Figure 2. Presumed concentric rings of sap flux movement inside the tree.

When illustrated, the t_{max} values indicate how the tree responds to its environment. Figures 3 and 4 illustrate t_{max} values for *P. pterocarpum* and *A. auriculiformis* respectively, during March 1994. *P. pterocarpum* (Figure 3) responds very rapidly to the onset of light, almost reaching its maximum sap flux in two hours. In addition, all six microbead thermistors register a response. This indicates that sap flux occurs to a depth of 6 cm, possibly more, and that sap flux for all 6 concentric rings must therefore be calculated. A lateral cut through *P. pterocarpum* shows it to be largely composed of sapwood and confirms that sap flux can occur in all the 6 concentric rings that are measured.

A. auriculiformis (Figure 4) responds differently to *P. pterocarpum*. Its t_{max} graph indicates that only the two outermost rings of the tree are conveying sap as indicated by thermistors 1 and 2. This is again evident from a cross-section of the tree, which is largely composed of heartwood, apart from the exterior 1 or 2 cm. The t_{max} values indicate lower sap velocity and the curves are less pronounced. For these reasons it can be expected that *A. auriculiformis* transpires less than *P. pterocarpum.*

The t_{max} values indicate which rings of the tree transpire and during which hours of the day they transpire. Having determined this, it is possible to calculate the volumetric flow of sap in the tree. Sap flux in *P. peltophorum* starts rather abruptly from 0.7 litres between 7.25 h and 8.25 h to 7.2 litres between 8.25 h and 9.25 h (Figure 5). Maximum sap flux occurs between 13.25 h and 14.25 h and is about 12.4 litres. The outermost ring monitored by thermistor 1 conveys the most water during the day (21.8 litres), whilst thermistors 5 and 6 monitoring the innermost rings of the tree show similar sap fluxes of 5.5 and 5.7 litres per day. The total sap flux on March 14 is about 84.6 litres.

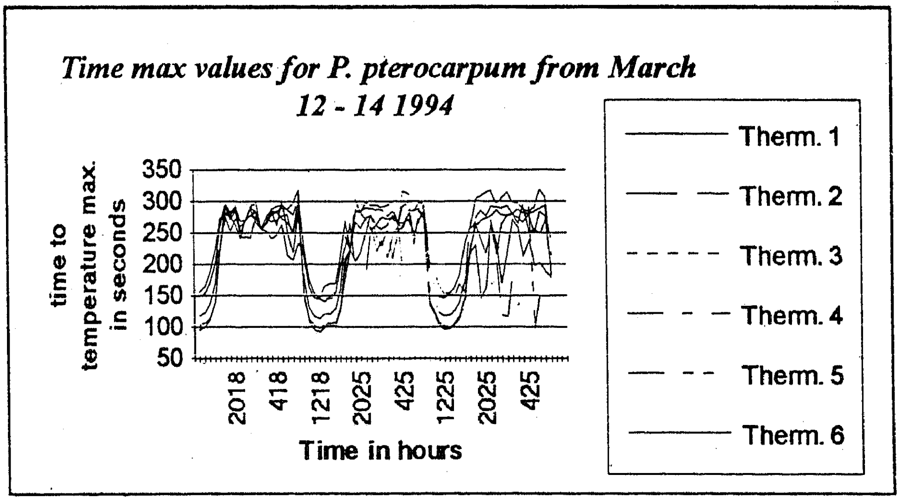


Figure 3. t_{max} values for *P. pterocarpum* in March 1994.

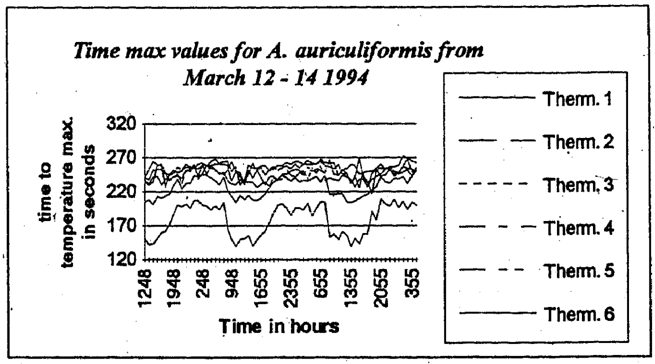


Figure 4. t_{max} values for *A. auriculiformis* in March 1994.

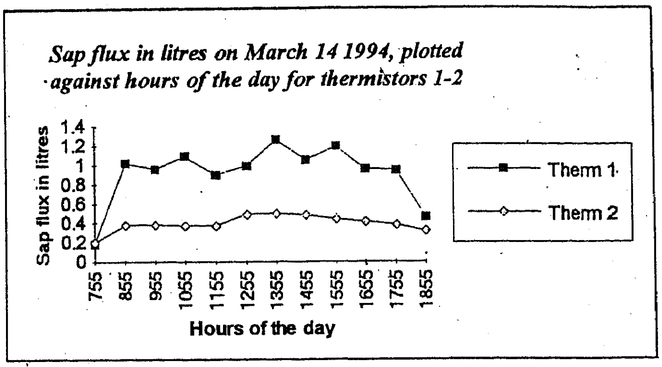


Figure 6. Sap flux for *A. auriculiformis* on March 14, 1994.

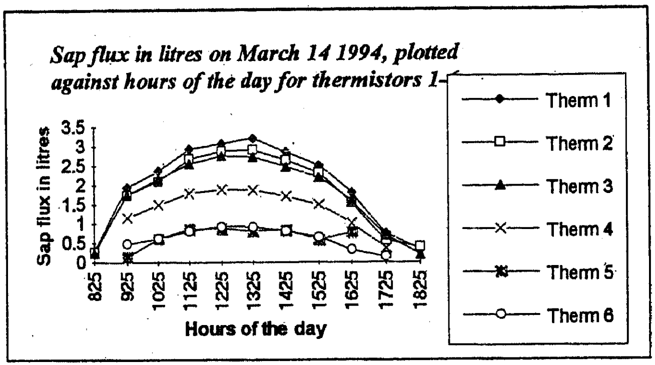


Figure 5. Sap flux for *P. pterocarpum* on March 14, 1994.

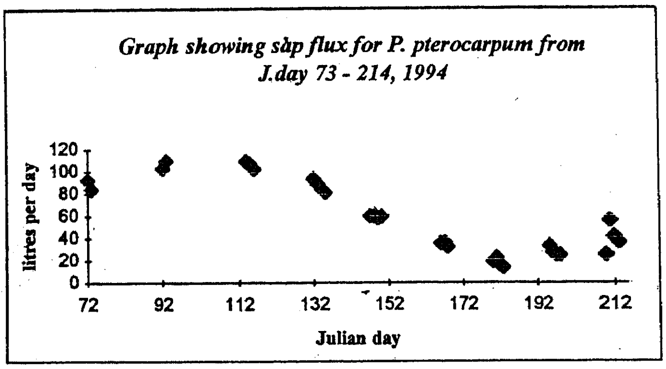


Figure 7. Sap flux for *P. pterocarpum* between March and July 1994. Each square indicates daily sap flux.

The case is rather different for *A. auriculiformis* (Figure 6) as only the 1st and 2nd thermistors indicate sap flux. The 4 inner rings show 'diffusivity' (the movement of heat through the woody material, without convective transport by sap) and indicate zero or very low sap flux. The calculations for sap flux therefore need to be made only for the outer 2 rings monitored by the 1st and 2nd microbead thermistors. It is evident that in *A. auriculiformis*, sap velocity is much lower than in *P. pterocarpum*. The curve is correspondingly less pronounced, showing that it is less responsive to light. Sap flux commences at about 7.55 h and during the first hour is about 0.9 litres. It reaches a maximum of 1.9 litres between 13.55 h and 14.55 h, declining to a stop at about 18.55 h. Total sap flux for 24 hours is about 16 litres.

The presence or absence of sunlight stimulates sap flux, particularly in *P. peltophorum* which is literally 'switched' on and off by sunlight, exhibiting a strong biological rhythm. Other variables, however, are also important in influencing sap flux, notably soil moisture and the physiological status of the tree (i.e. whether it is in leaf or bare).

Figures 7 and 8 show total daily sap fluxes for *P. pterocarpum* and *A. auriculiformis*, from mid March to July (Julian days 73–214). In *P. pterocarpum* (Figure 7) sap flux increases from about 85 litres per day in mid March (Julian day 73) to about 110 litres per day by the latter half of April (Julian day 112). This is partially attributable to increasing leaf cover density (*P. pterocarpum* loses its leaves between December and February, but by February, March and April is in the process of leafing again), and the greater transpirative surface that is thereby created. Additionally, transpirative demand is also increased, as summer progresses and net radiation and dry bulb temperature rise. Sap flux therefore increases to whatever extent is permitted by the level of moisture in the soil. Thereafter total daily sap flux declines steadily due to decreasing soil moisture, as the rainless days of summer progress and the available soil moisture evaporates, percolates or is transpired by the tree. By the end of June (Julian day 181), sap flux is reduced to about 15 litres per day. After this, it starts to increase again, as the first rains of the SW summer monsoon augment soil moisture.

The pattern is different for *A. auriculiformis* (Figure 8). As the tree is almost evergreen, there is little influence of increasing leaf density on sap flux. Instead, there is a steady decline from 14 litres per day in mid March (Julian day 72), to about 4 litres per day by the end of April (Julian day 112), as soil moisture brought by the 1993 NE winter monsoon declines. This basic level of sap flux remains unaffected by the first showers of the 1994 SW monsoon that started on Julian day 160.

Figures 9 and 10 show how these two trees respond to a sudden increase in soil moisture caused by an 11 mm rainfall at about 21.00 h on Julian day 210 (July 29). The $t_{\max}$ values for *P. pterocarpum* (Figure 9) on Julian day 210 before the rain falls, indicate maximum sap velocity at 14.03 h with a minimum $t_{\max}$ value of 166 sec. After the rain, the $t_{\max}$ values on Julian day 211 decrease from 166 sec to 122 sec at 12.09 h on Julian day 211, indicating a dramatic increase in the velocity of the sap. Of further note is that the 5th and 6th thermistors (5 and 6 cm deep) do not indicate sap flux during Julian day 210. However, the rain on Julian day 210 allows the 5th thermistor to start conveying sap once again on Julian days 211 and 212. (Note that during March when soil moisture was high, all 6 thermistors were indicating sap flux (Figure 3).) The decrease in $t_{\max}$ values at 21.06 h on Julian day 210 indicates that the tree might have absorbed water at night and that some sap flux is perhaps taking place although there is no transpiration. This may be due to an increase in the soil moisture potential after the rainfall, which has allowed water to move into the tree. The effect of the rain is short lived, as a steady decrease in sap flux is indicated by the increasing $t_{\max}$ values for Julian days 212 and 213. By Julian day 213, the 5th thermistor once again indicates absence of sap flux altogether.

A. auriculiformis (Figure 10), on the other hand, shows no such response to the rainfall on Julian day 210. Only the first thermistor shows a slight decrease in $t_{\max}$ values from around 180 sec on Julian day 210, to about 175 sec on Julian day 211, almost negligible when sap flux is calculated. The $t_{\max}$ values seem to show that sap flux is altogether absent, with only thermal 'diffusivity' occurring. Calculations with these values, however, still indicate small quantities of sap flux (Figure 8).

It should be emphasized that the discussion above revolves around only 2 of the 8 trees studied (1 for each species), and that intra-species $t_{\max}$ values (and thus intra-species sap fluxes) are not necessarily identical to the two examples used. This is illustrated in Table 1 which summarizes the total monthly sap fluxes (in litres) of all four *A. auriculiformis* and all four *P. pterocarpum* monitored between March and July 1994. The trees examined in the discussion above are indicated in the table as Aa3 and Pp3. The average sap flux for *P. pterocarpum* over the 5 months of measurements comes to 8.1 m^3, whilst for *A. auriculiformis*, average sap flux over 5 months comes to 2.3 m^3.

From the data in Table 1, it is evident that all four *A. auriculiformis* together transpired about 9.4 m^3 of water between March and July 1994, whilst all four *P. pterocarpum* together transpired about 32.6 m^3 of water over the same period. Rainfall in Auroville in 1994 was 1.15 m^3 per m^2. Sap flux between March and July for *A. auriculiformis* as a percentage of the rainfall received

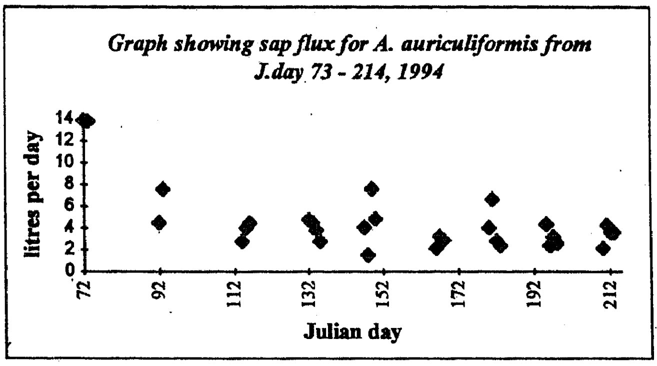


Figure 8. Sap flux for *A. auriculiformis* between March and July 1994.

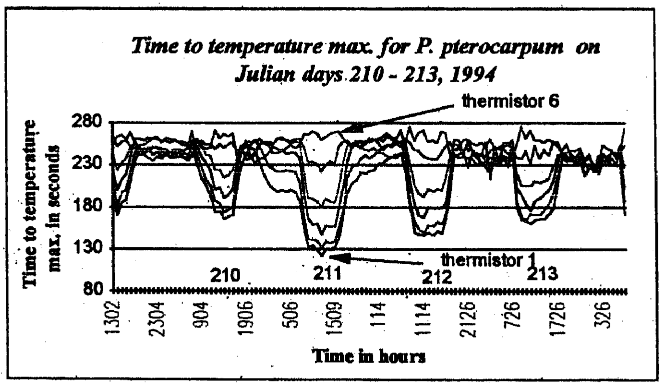


Figure 9. $t_{\max}$ values for *P. pterocarpum* in July 1994.

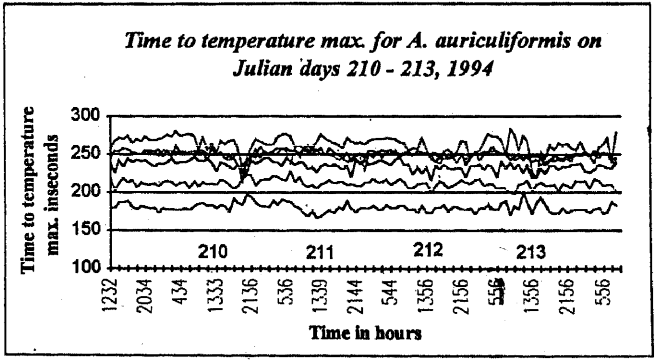


Figure 10. $t_{\max}$ values for *A. auriculiformis* in July 1994.

Table 1. Total monthly sap fluxes for the eight trees monitored between March and July 1994

Tree no.	March (in litres)	April (in litres)	May (in litres)	June (in litres)	July (in litres)	For 5 months (in m^3)
Aal	597	511	398	186	159	1.85
Aa2	745	753	321	381	237	2.44
Aa3	705	429	405	306	189	2.05
Aa4	1334	954	300	291	159	3.04
Average	845	662	356	291	186	2.34
Pp1	1338	2192	1326	1326	1329	7.82
Pp2	450	938	750	282	264	2.68
Pp3	2751	3568	2625	720	912	10.58
Pp4	1410	3128	3045	1701	2198	11.48
Average	1487	2457	1937	1007	1176	8.14

upon the canopy area of all eight trees (276 m^2) is therefore about 2.9%. For *P. pterocarpum* the figure is about 10.4%. During the five-month study, the eight trees together transpired about 13.3% of the rainfall that fell upon their canopies in 1994.

Assuming that the seven unmeasured months might give equivalent values, these eight trees would, during 1994, transpire 31.1% of the rainfall falling on their canopies. This figure is naturally unrealistic. *P. pterocarpum*, for example, sheds its leaves during winter putting a stop to transpiration altogether, whilst soil moisture and transpirative demand undergo extensive changes during the monsoons.

As runoff from the research plot is minimal, it can be assumed that the 68.9% of the rain not transpired by the trees infiltrates into the soil and percolates. What proportion of this reaches groundwater and what proportion of this is evaporated directly from the soil or is transpired by understorey vegetation remains to be seen.

Attempts to quantify the hydrological impact of reforestation in Auroville on the water balance of the local soil and groundwater should ideally include measuring runoff and evaporation from 'bare' as well as reforested areas. It is also important to evaluate with advanced research methods, what proportion of the sap flux comes directly from groundwater sources and what proportion of it comes from soil moisture[3].

In conclusion, the study has allowed sap fluxes in two tree species, *P. pterocarpum* and *A. auriculiformis*, to be quantified, showing that sap flux in *P. pterocarpum* is significantly higher than in *A. auriculiformis* (Table 1). Changes in daily sap flux between March and July 1994, were evident for both *P. pterocarpum* (Figure 7) and *A. auriculiformis* (Figure 8). In *P. pterocarpum* the onset of the SW monsoons stimulated an increase in sap flux. In *A. auriculiformis* no such response occurred presumably because the necessary soil moisture level had not yet been attained by the time measurements ended in July.

To some extent the study has also unveiled the inner dynamics of water movement in *P. pterocarpum* and *A. auriculiformis*. When soil moisture was plentiful, sap flux in *P. pterocarpum* occurred to a depth of 6 cm (Figure 3) and in all 6 concentric rings (Figure 2), whilst in *A. auriculiformis* sap flux occurred to a depth of only 2 cm (Figure 4) and was confined to the outer 2 concentric rings (Figure 2). This pattern changed as soil moisture decreased during the rainless summer months. In *P. pterocarpum*, sap flux stopped in the 2 innermost concentric rings and occurred in only the 4 outermost concentric rings (Figure 9). In *A. auriculiformis*, only thermal 'diffusivity' in the 2 outer sap conveying rings occurred during the dry summer months (Figure 10), indicating that ideally more sensitive equipment is needed to measure sap flux at such low velocities.

With further research on other tree species, it will be possible to know how reforestation as a land use compares with agricultural or domestic land use, or with just leaving the ground bare. In particular, it will be possible to know which trees and how many trees to introduce into which hydrological situations. In an area with scarce water resources for example, it might be more beneficial to introduce trees which are frugal users of water, like *A. auriculiformis*, whilst trees with high transpirative capabilities like *P. pterocarpum* might be better utilized in areas prone to water logging. Concerning Auroville and coastal areas in general, where urbanization and population are rapidly increasing and salt water intrusion and falling groundwater levels threaten to bring environmental catastrophe, trees selected for their transpirative characteristics and planted in strategically chosen areas could do much to redress the hydrological balance.

Appendix 1

Marshall's formula as modified by Cohen *et al.*[2] is quoted below:

'The temperature elevation, T, produced by the heat pulse after time, t, and at a distance $r = (x^2 + y^2)^{0.5}$ from the line heater, is given by (Marshall[1]).

$$T = H/4\pi pckt \, \exp((x-Vt)^2 + y^2)/4kt, \qquad (1)$$

where H is the heat output per unit length of the heater, and p, c, and k are the density, specific heat, and the thermal diffusivity of wet wood, respectively. The heat wave convective velocity, V, is defined as:

$$V = (p_1 c_1 / pc) J_1 \, . \qquad (2)$$

The function defined by equation (1) has a maximum occurring at $t_{\max}$ when the first derivative of eq. (1) is equal to zero. This condition yields,

$$V=(r^2-4kt_{\max})^{0.5}/t_{\max}. \quad (3)$$

For a given value of r, and with known physical and thermal properties of the live wood, the measurement of $t_{\max}$ in eq. (3) allows the computation of the water flux J_1, in eq. (2). The only property that is difficult to determine is the thermal diffusivity, k of the live wood. With $V=0$, eq. (3) yields,

$$k=r^2/4t_{\max}. \quad (4)$$

Consequently, k can be determined when no convective transport is taking place. In order to determine the volumetric flow, F, the water flux, J_1 must be integrated over the cross-sectional area of the stem,

$$F=\int J_1 ds, \quad (5)$$

where ds is the element of stem area in which J_l has been determined.'

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Bioleaching of copper from chalcopyrite ores coated with polyaniline film

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A simple method of extracting copper and iron from chalcopyrite ore using the bacterium *Thiobacillus ferrooxidans* is reported. By surface modification of the ore and using a thin polyaniline film, the rate of bioleaching can be increased considerably. The film acts as a coating to the heterogeneous ore surface, leaving the catalysable sites more frequently exposed to microbial attack. The inhibitory effect is due to formation of jarosites, and is found reduced in the coated ore samples. This may be due to ferric hydroxide formed within the film coat during leaching.

SEVERAL reports pertaining to improvement of bioleaching process have appeared in the literature. The effect of detergents[1,2] and passing of mild electric current[3,4] etc. have been reported to be beneficial for the recovery process. On the other hand, the adverse effect of detergents[5] has also been reported. Leaching primarily involves a direct pathway[6–9], wherein *Thiobacillus ferrooxidans* attacks the ore mineral causing leaching. Thus an alteration of the ore–bacteria interface would affect leaching rate either positively or negatively. Murr and Berry[10] observed no preferential adhesion of cells of *T. ferrooxidans* to specific surface features such as steps or dislocations, needed both for molybdenum sulphide and chalcopyrite. However, higher growth rate was observed at the inclusions. As the surface of the ore dictates the rate-determining factors of leaching, in the present work premise the dislocation sites were artificially exposed to microbial attack. Another reason for following this procedure was that gangue material containing oxides of magnesium, calcium and aluminium may be inaccessible to microbial populations, and by preventing the catalytically inactive growth, one could arrest the extent of precipitation of jarosites (precipitation of ferric hydroxide complex associated with growth of *T. ferrooxidans*).

The microorganism, *T. ferrooxidans*, is a chemolithotrophic bacteria deriving its energy from ferrous oxidation reaction utilizing carbon dioxide as the main carbon source. The strain of *T. ferrooxidans* in ATCC19859d (supplied by Dr David Holmes, USA) was repeatedly subcultured in 9 k medium[11] (ammonium sulphate, 3 g;

Table 1. Composition (proportion-wise) of polyaniline coated chalcopyrite ore samples

Sample	Aniline (M)	Ammonium persulphate (M)	Chalcopyrite ore (g)
1	0.1	0.73000	0.5
2	0.1	0.07300	0.5
3	0.1	0.00073	0.5
4	0.5	0.07300	0.5

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*For correspondence

potassium chloride, 100 mg; dipotassium hydrogen phosphate, 500 mg; magnesium sulphate, 500 mg; with pH adjusted to 1.8 + 30 g ferrous sulphate added to it, and the pH adjusted to 2.0). The ore sample, chalcopyrite concentrate, was a gift from M/s Hindustan Copper Ltd.

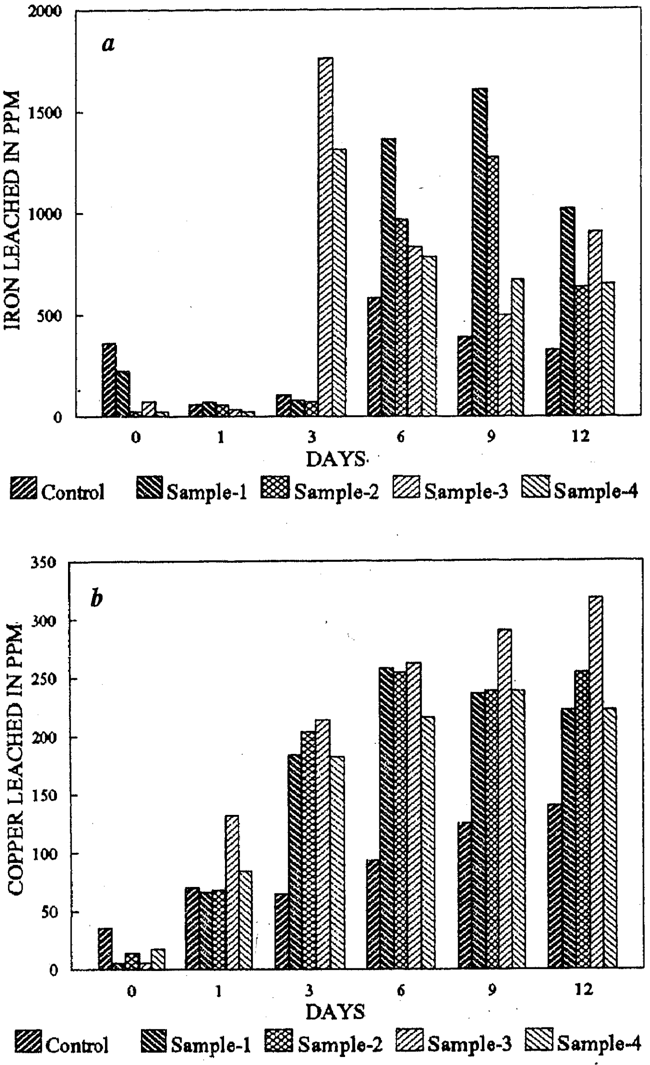

Figure 1. Comparative amounts of leaching of iron (*a*) and copper (*b*) from polymer coated and uncoated chalcopyrite ore samples up to 12 days of studies. Polymer-coated sample 3 is found most suitable, releasing maximum amount of iron (3rd day) and copper (12th day). All other polymer-coated samples also have higher efficiency of leaching metals than that of control.

Leaching was studied using 100 mg of cell concentrate obtained from well grown culture of *T. ferrooxidans* at pH 2.0 and 1 g of ore/sample (surface modified ore). The procedure was then repeated by adding 9 k medium instead of the cell culture. The samples were subjected to continuous shaking for optimal aeration at 30°C in a tissue culture shaker at 12 rpm.

For the assay of copper and iron, 0.5 ml of aliquots was collected at 24 h intervals and subjected to centrifugation at 7000 rpm for 10 min at room temperature using a Remi microfuge. The pellet was discarded and the supernatent diluted 200 times for measurement in

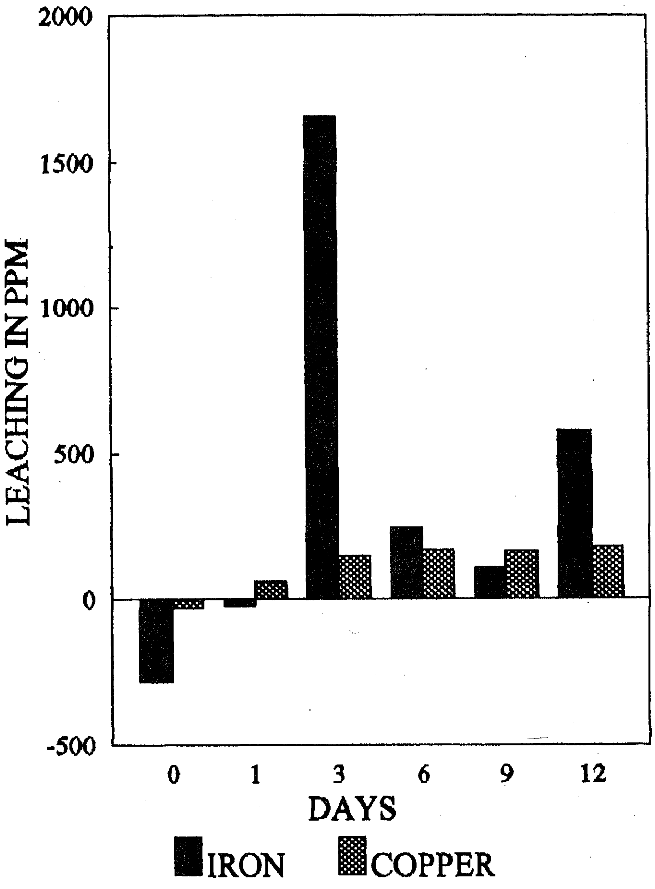

Figure 2. Comparative bar diagram representation for copper vs iron leaching from polymer-coated sample 2 in relation to control as a function of days.

Table 2. Comparative data of copper vs iron leaching from control and polymer-coated chalcopyrite ore sample as a function of days

	Control		Sample 1		Sample 2		Sample 3		Sample 4		Comp. data (sample 2)	
Days	Iron	Copper	Iron	Copper	Iron	Copper	Iron	Copper	Iron	Coppe.	Iron	Copper
0	360.0	36	223.3	6.0	23.3	14.0	76.7	6.0	23.33	18.00	– 336.67	– 22.00
1	60.2	70.2	73.3	66.0	56.7	68.0	36.7	132.0	23.33	84.00	– 3.53	– 2.20
3	107.3	64.6	80.0	184.0	76.7	204.0	1763.3	214.0	1310.00	182.00	– 30.60	139.40
6	584.5	93.2	1363.3	258.0	966.7	254.0	830.0	262.0	783.33	216.00	382.13	160.80
9	391.8	125.8	1606.7	236.0	1273.3	238.0	500.0	290.0	673.33	238.00	881.53	112.20
12	325.7	140.4	1020.0	222.0	636.7	254.0	906.7	318.0	650.00	222.00	310.93	113.60

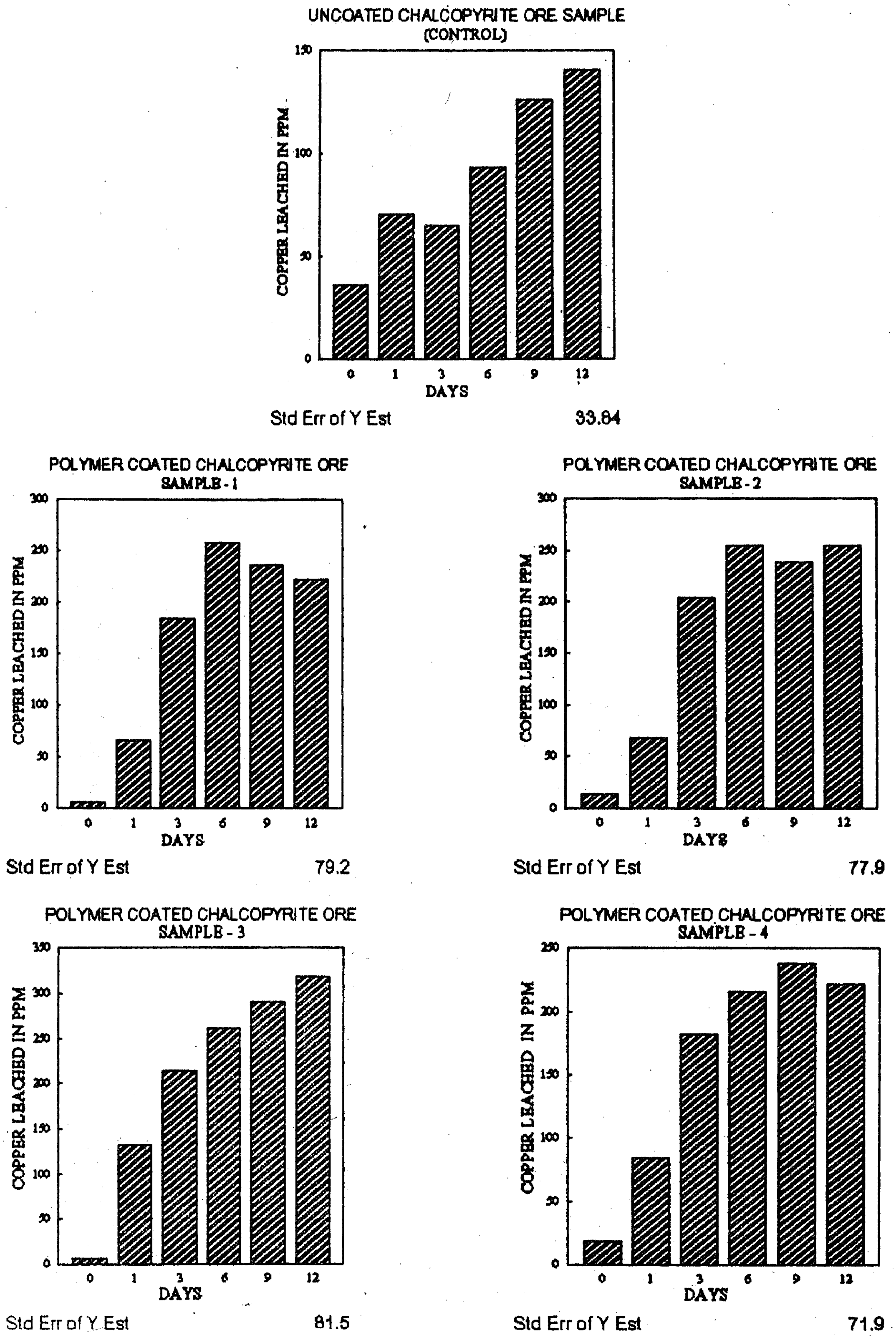

Figure 3 *a*. Bioleaching of copper from polyaniline-coated and uncoated chalcopyrite ore samples by *T. ferrooxidans* and the standard error of estimation during studies up to 12 days.

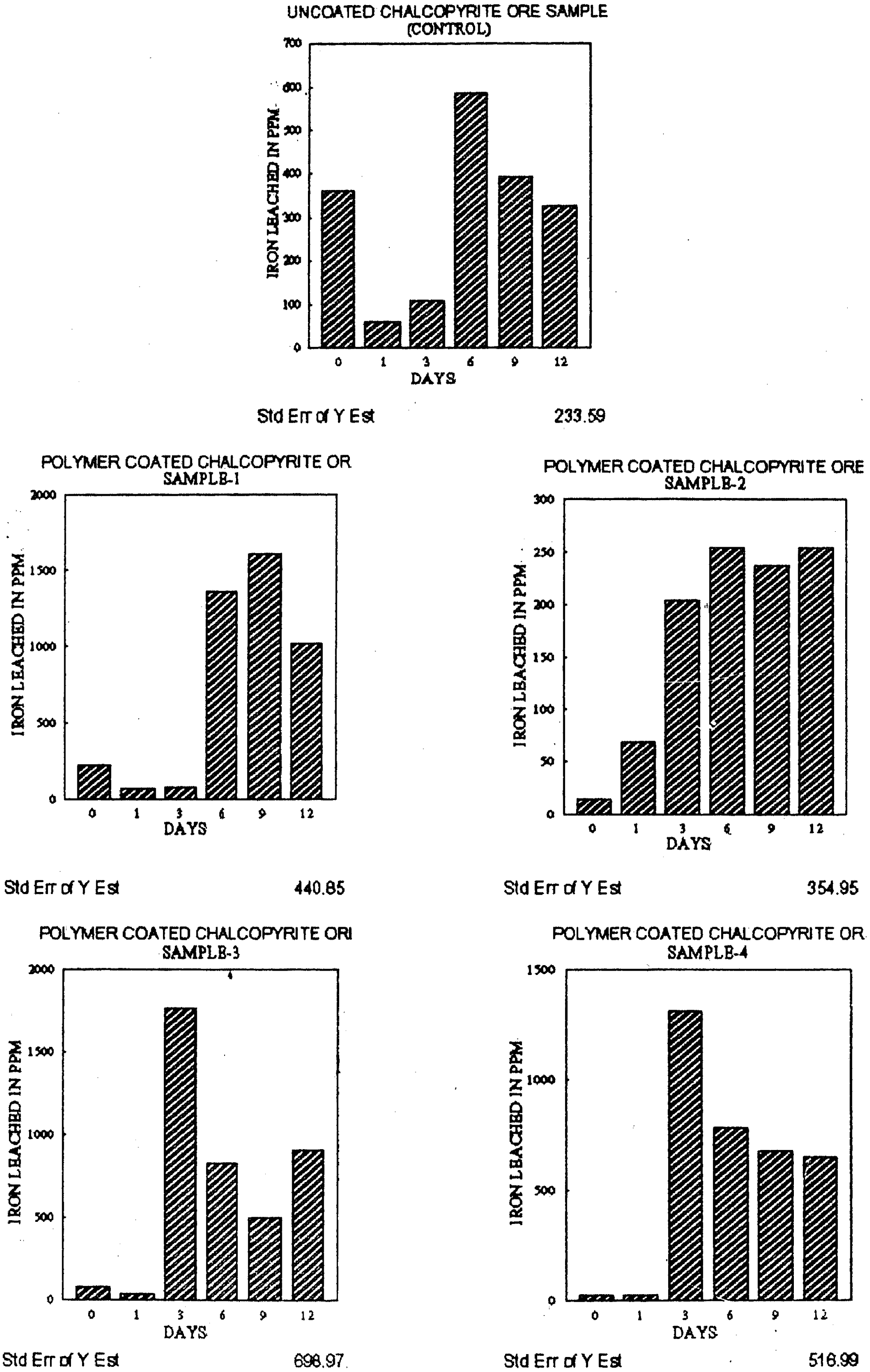

Figure 3*b*. Bioleaching of iron from polyaniline-coated and uncoated chalcopyrite ore samples by *T. ferrooxidans* and the standard error of estimation during studies up to 12 days.

an atomic absorption spectrophotometer (model: Varian AA-1475). For reliable measurements five replica samples were taken for each aliquot and both the iron and copper concentrations were measured.

The ore surface was modified by depositing a thin film of polyaniline polymer. The thickness of the film was varied by adjusting the concentration of the oxidant (ammonium persulphate) and the monomer (aniline). In each case the system was allowed to reach equilibrium. The ore coated with the polymer was filtered and washed with distilled water. The dried sample was then tested for leaching. The four different samples were obtained by varying the relative concentrations of the oxidant and the monomer (see Table 1). The leaching experiment was performed with the aforesaid samples and the original ore.

A comparison of leaching of copper and iron both in the polymer-coated ore samples and in the uncoated ore samples in their naive form is shown in Figures 1 and 2. As can be seen, the polymer-coated samples leach copper at a faster rate. To prevent possible errors resulting from the heterogeneous nature of the different fractions of the ore samples, the kinetic profile was normalized taking five replicas from aliquotes of each sample at each day point. The percentage error in the measurement is shown by the bar diagram (Figure 3 *a* and *b*). As described earlier, leaching from each of the samples was studied both in the presence of and absence of *T. ferrooxidans*. Leaching of Cu from the polymer-coated samples was always higher in the presence of bacteria (Figure 1), indicating that microbial attack was possible despite the presence of polymer coating and that the enhanced leaching observed was not entirely due to alteration of the chemical leaching profile.

Figure 1 shows the iron-leaching profile of the original ore sample and that coated with polymer. As is seen, for samples coated with the polyaniline, the solution affinity for iron is higher in the initial stage (3–5 days) but falls subsequently. Figure 1 is a typical example of how the leaching of Cu and Fe took place from polymer-coated ore sample.

In all the four ore samples coated with polymer, leaching of copper and iron was higher in the presence of *T. ferrooxidans* compared to control samples (without bacteria). This indicated that biocatalysis operates even in polymer-coated states. Figures 1–3 also illustrate the time profiles of copper and iron respectively appearing in the solution. It is clear that leaching rate was higher for polymer-coated chalcopyrite samples compared to that of uncoated sample. The recovery of copper recorded every day was steady for each coated sample, whereas iron underwent fluctuations during leaching. This was due to precipitation of ferric hydroxide as jarosite. Among the polymer-coated samples, sample 3 was found more effective in both the categories of metal leaching (Table 2). This suggested that for sample 3, coating by polymer helped in maximum recovery. In the initial phase of experiment, due to direct leaching by concentrated sulphuric acid, the uncoated ore sample (control) always had higher concentration of copper or iron compared to that of any polymer-coated sample. Comparative results of leaching on the first two days show that sample 3 was completely free from acid leaching, thus proving that polymer coating of sample 3 was complete and assisted in better leaching. The composition of polyaniline polymer (Table 1) indicates that a thin film of polyaniline polymer covering the whole chalcopyrite ore particle might be most suitable for effective bacterial attack on the coated ore sample. It prevents ferric hydroxide precipitation and other metabolites which could cause a change in the constituents and conditions of medium acting as a deterrant in the growth of bacteria or leaching of the metal.

A number of surface-reacting agents presently being used for improvement of leaching have shown a negative effect on the bacteria. The present technique which involved modification of ore surface by covering the ore by a polyaniline film enables the organism to attack the encapsulated ore and ensures the protection of unwanted metabolites from interfering in the composition of the medium by stopping leakages through the polymer film. Unlike the surface reacting agents, this method is useful to all micro-organisms.

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On the occurrence of manganese micronodules in the deep-sea sediment west of Lakshadweep Ridge, Arabian Sea

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Sediment cores collected from the eastern Arabian Sea, west of Lakshadweep Ridge at water depths of 2800–4400 m, have indicated the presence of manganese micronodules over an area of 0.08 million sq km. These nodules occur sparsely in the sediments between 10 and 50 cm below the seafloor. The host sediment is composed of brown to dark brown pelagic clay of 5 to 20 cm thick. The nodules occur as thin coatings and infillings of Mn-hydroxide mainly over planktonic foraminiferal tests. The micronodules do not exceed 0.5% by weight of the host sediment but are abundant in the coarse fraction (> 53 μm), constituting 2.6 to 24%. Birnessite is the main Mn-hydroxide mineral in the nodule. Chemical data suggest that micronodules are Mn-rich, and are generally Fe-depleted with Mn/Fe ratio varying between 4.76 and 80. Micronodules have highly variable trace element concentrations. In contrast to micronodules of Central Indian Basin, the present nodules are depleted in trace elements, though both have more or less comparable Fe–Mn abundance.

Occurrence of ferromanganese micronodules in surface and sub-surface sediments has been described from both marine and lacustrine regimes the world over. The formation of both macro and micronodules demands several favourable conditions such as low sedimentation rate, an oxidizing environment, presence of nucleating material, influence of microbiotic activity and the availability of elements for precipitation from lithogenous component and/or pore water. These conditions facilitate the growth of colloidal aggregates of Mn–Fe oxides and hydroxides ranging from microscopic specks to macroscopic concretions[1]. While authigenising Mn and Fe, the nodules also incorporate traces of Ni, Cu, Co, Pb, Zn, noble metals, elements of platinum group and REE.

The basic difference between manganese macro- and micronodule is their size; the latter is commonly smaller than 1 mm and the former can be as large as a few cm (ref. 2). Micronodules from the Pacific are relatively well documented. The distribution of Fe, Mn, Cu, Ni and Co in coexisting Mn-nodules and micronodules from the world's oceans indicates that in about 60% of the coexisting pairs, micronodules are found to show relatively low Ni, Co and Cu and a low Mn/Fe ratio[3]. The morphology characteristics[4] and geochemistry[5–7] of micronodules from the Pacific have been extensively studied. Micronodules of the Central Indian Basin (CIB) have also been studied in recent years. Their distribution, morphology and geochemistry have been dealt by many workers[8–10]. Occurrence of micronodules has been recently reported from Bay of Bengal also[11,12].

The present work is based on preliminary studies of core samples collected from the western part of the Lakshadweep Ridge (LR) during cruises of *RV Samudra Manthan* (RVSM) of the Geological Survey of India between 1986 and 1993. Many of these cores collected from depths of 2800–4400 m have indicated the presence of manganese micronodules in the sub-bottom sediment layer. This layer is composed of brown to dark brown pelagic clay and forms the host sediment. Possibly this is the first report of the occurrence of micronodules from this part of Arabian Sea. The objective of the paper is to present basic data on mode of occurrence, morphology and geochemistry of these micronodules and discuss their probable origin.

Sub-bottom samples of 27 RVSM gravity and piston cores (Figure 1) were partly used for the present study. About 25–30 g of the host sediment was treated with 50 ml of 15% H_2O_2 for 15–20 min to rapidly separate the micronodules from the matrix. MnO_2 of the sediment being a natural catalyst splits H_2O_2 into H_2O and O_2 (ref. 13). The reaction is exothermic and the intensity of heat and fumes generated is an indication of the abundance of micronodules in the sediment. After gently stirring and washing, the slurry was wet-sieved using + 230/270 ASTM sieve to separate the coarse fraction,

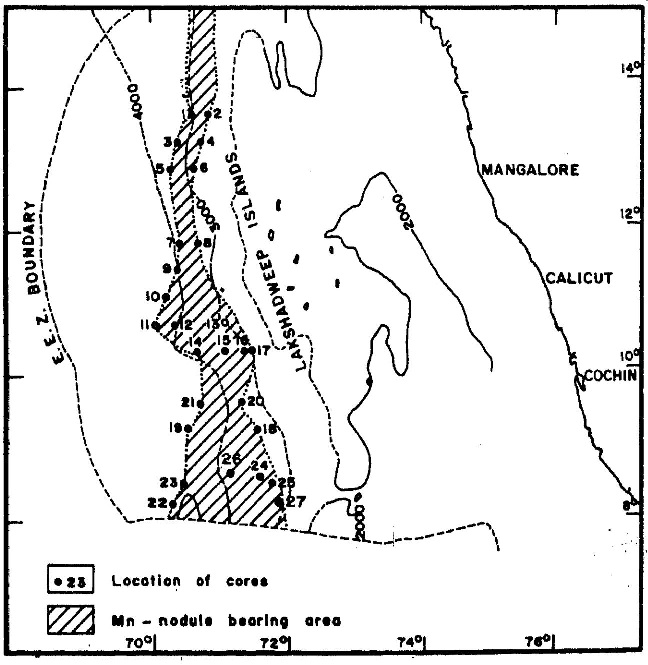

Figure 1. Map of the eastern Arabian Sea showing manganese micronodule-bearing area with locations of gravity cores.

which was dried, and then treated with 20 ml of 10% acetic acid for 10–15 min to remove the uncoated carbonate tests. The concentrate thus obtained was dried, weighed and examined under microscope to find the non-carbonate impurities. In few cases, the coarse fraction was subjected to heavy liquid separation using bromoform (sp. gr. 2.8). The thinly or partially coated micronodules floating in the heavy liquid were collected to study their morphology. Siliceous tests and detrital minerals in the concentrate were less than 1%.

The chemical analysis was carried out on 12 out of 27 concentrates. About 0.1 g of the concentrate was treated with 5 ml aqua-regia and then with 1 ml of 40% HF. The solution was gently evaporated to dryness and 5 ml of concentrated HCl was added and again heated to dryness. After another addition of 5 ml of 1 : 1 HCl, the solution was gently warmed. Mn, Cu, Pb, Zn, Ni and Co were determined using AAS (Varian Spectr. AA 30) in air–acetylene flame whereas Fe was determined in nitrous oxide–acetylene flame. Necessary dilutions were made before feeding the solution into AAS. Calibration standards used were prepared from recommended metals/salts.

X-ray diffraction of one nodule sample (core no. 5057; location 26 in Figure 1) was carried out on Phillips APD refractometer using CuK$_\alpha$ radiation to know the mineralogical components of the nodule. Results were cross-checked using FeK$_\alpha$ radiation.

The micronodules are distributed over a length of approximately 800 km between 8° and 15°N latitudes and 69° and 72°E longitudes. The area of their distribution is narrow (~ 45 km) in the north but gradually widens towards south (~ 380 km) covering nearly 0.08 million sq km of the eastern Arabian Sea (Figure 1). The middle and lower parts of the slope and the areas fringing the abyssal plain show greater abundance of nodules.

Mn-micronodules occur at depths 10–50 cm below sea bed. The host sediment varies in thickness from 5 to 20 cm and is a distinct layer of brown to dark brown (10YR 5/3–4/3) pelagic clay. It occurs between pale brown hemipelagic clay above and olive grey calcareous ooze/hemipelagic clay below, indicating respectively oxic and reducing conditions. Preliminary studies reveal that the micronodules constitute not more than 0.5% by weight of the host sediment, but they are abundant in the coarse fraction (> 0.05 mm), constituting 2.6 to 24%. Interestingly, one core (No. 5050, location 27 in Figure 1) from far-south reveals the presence of fossil Mn-oxidation front even below 50 cm level. The litholog of a representative core (No. 2631, location 15 in Figure 1) from the central part is given in Figure 2.

The micronodules occur as jet black to brownish black coatings and infillings essentially over microforaminiferal tests, less commonly over skeletal tests of pteropods and radiolarians and rarely over glass shards. Authigenic coating over planktonic tests is the most dominant type. The nodules display spheroidal, elongated, botryoidal, tubular or pseudo-framboidal morphologies and commonly retain the shape of skeletal tests or substrate (Figure 3). Their size is generally < 0.25 mm, but larger ones are not infrequent. Pteropod-coated nodules can be even 2 mm long. Partial or incipient coating of the nucleii is seen more in the northern part of the area. The surface texture of the micronodules is characterized by irregular, gritty and at times smooth and polished appearance with bright lustre. Growth of secondary minerals over the nodule often masks its colour and texture.

XRD result suggests birnessite as the major mineral phase in the present micronodule. This is the intermediate variety of the common manganese hydroxides in Mn-nodules and normally forms under suboxic condition. Associated minerals detected are quartz, feldspar, chlorite and illite.

Chemical data of 12 samples are given in Table 1. The data suggest that Mn content is high, varying between 25.10 and 43.0% with an average of 35.83%. Fe varies from 0.53 to 5.27% with an average of 1.82%. The Mn/Fe ratio varies considerably from 4.76 to 80 with an average of 31.10. The trace element content is as follows; Cu: 178–1,100 ppm, Ni: 20–2,000 ppm, Zn: 28–350 ppm, Co: 10–300 ppm and Pb: 40–1,600 ppm. In general, higher Mn values are recorded from deeper areas. The chemistry of the present occurrence and that

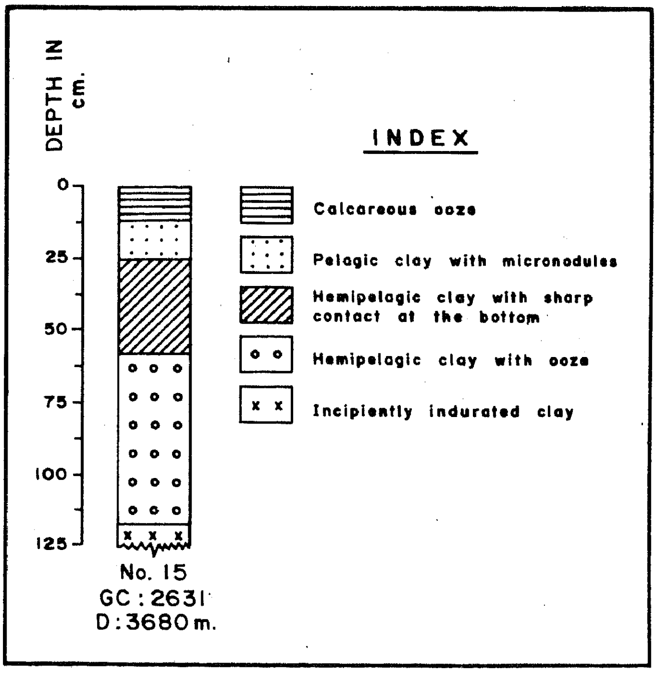
DEPTH IN cm.
0
25
50
75
100
125
No. 15
GC : 2631
D : 3680 m.
INDEX
Calcareous ooze
Pelagic clay with micronodules
Hemipelagic clay with sharp contact at the bottom
Hemipelagic clay with ooze
Incipiently indurated clay

Figure 2. Litholog of a representative core showing Mn-micronodules-bearing horizon.

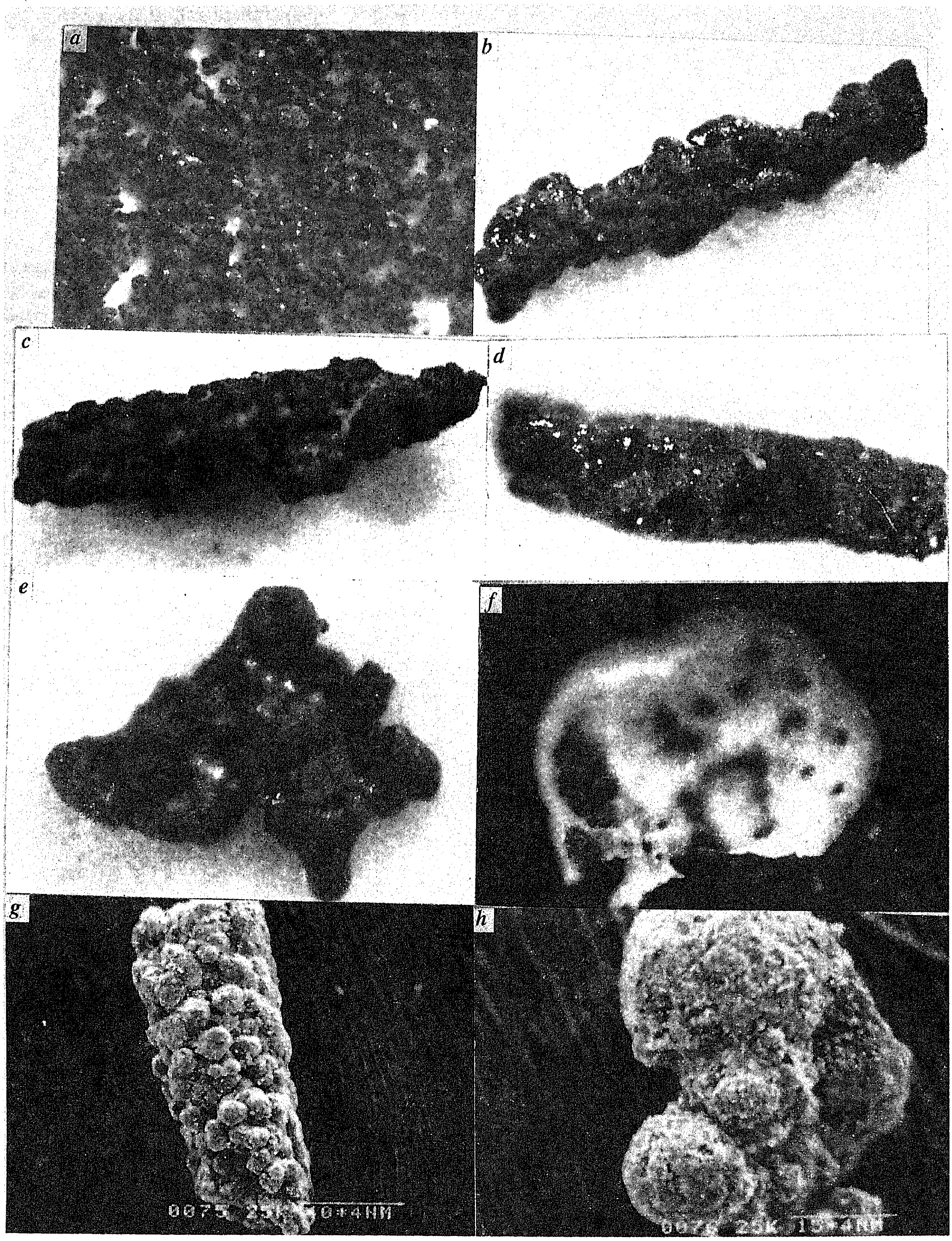

Figure 3. Photomicrographs of various types of Mn-micronodules: ***a***, spherical/tubular (assorted), scale (× 80); ***b***, elongated botryoidal; ***c***, tubular; ***d***, tubular gritty; ***e***, semipolished and polished with luster, (***b*** to ***e*** scale (× 250)); ***f*** incipient coating over *G. menardii*. SEM photos (× 150): ***g***, fine hemispherical crystalline aggregates and ***h***, exhibiting poorly developed micro concretions.

of other known occurrences are given in Table 2 for comparison. Micronodules of the present area are broadly comparable to CIB micronodules in their Mn and Fe contents and Mn/Fe ratio. The trace element values in the latter[2], however, are significantly higher and the former is highly depleted of the same. In relation to the micronodules of Bay of Bengal[11,12], the present nodules have higher Mn and comparable Fe, Ni and Co contents.

Marine Mn-mineralization is controlled by three accretionary modes[11,12], viz. hydrogenous precipitation, oxic diagenesis and suboxic diagenesis. Triangular plots of Mn, Fe and (Cu + Ni + Co) × 10 contents of macro/micronodules have been used to delineate the environments of nodule precipitation[14,15,11]. The micronodules and Mn-coating of the present area fall within the suboxic environment (Figure 4). This is corroborated further by the disposition of the host sediment between lithologies indicative of oxic and reducing conditions. Similar sediments, with light coloured brown units above and olive coloured units below showing sharp colour boundary suggestive of an active oxidation front, have also been reported from the Madeira Abyssal Plain, N. Atlantic[16]. Brown colours in pelagites are commonly produced by concentrations of manganese hydroxides[17]. Higher Mn/Fe ratio implies an early diagenetic effect on nodule growth[2,9]. A transition from reducing condition

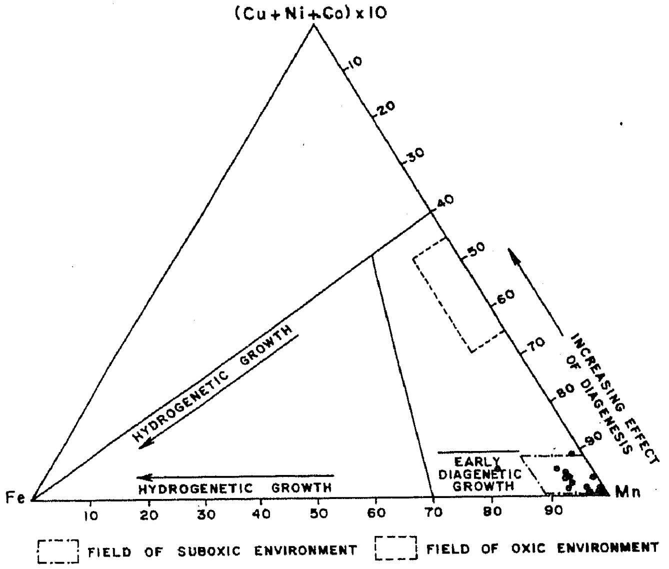

Figure 4. Plot of composition of micronodules from the area in Fe–Mn–(Cu + Ni + CO) × 10 triangular diagram. Most of the values fall in the field of suboxic environment.

Table 1. Mn, Fe, Cu, Ni, Zn, Co and Pb values with Mn/Fe ratio of 12 Mn-micronodule samples of the area with water depths

Location no.*/ core no.	Water depth (m)	Sub-bottom depth (cm)	Mn (%)	Fe (%)	Mn/Fe	Cu (ppm)	Ni (ppm)	Zn (ppm)	Co (ppm)	Pb (ppm)
4/2948	2901	23–26	25.10	5.27	4.76	400	1500	200	10	250
6/2939	2874	32–35	30.16	2.17	13.90	300	1500	270	200	170
12/PC32	4048	15–23	31.00	0.60	51.47	900	2000	350	300	220
14/2630	3784	32–36	40.00	0.60	66.67	490	650	150	260	150
15/2631	3680	13–26	33.10	2.00	16.55	430	765	285	125	125
18/3951	3508	13–22	31.00	1.85	16.76	225	750	ND	250	220
19/3953	4246	21–35	41.18	2.30	17.90	1100	1200	ND	230	ND
21/4563	4371	22–40	42.41	0.53	80.00	178	190	76	60	40
22/5051	4321	27–40	43.00	1.20	35.83	320	750	300	175	1600
23/5055	4246	44–50	41.00	1.20	34.17	270	20	28	35	150
24/5059	3613	21–33	39.00	2.00	19.50	390	1100	165	120	190
25/5060	3363	23–36	33.00	2.10	15.71	190	500	200	190	170
Average			35.83	1.82	31.10	433	910	202	163	295

*Location no. is shown in Figure 1.

Table 2. Average chemical composition of micronodules from Arabian Sea, Bay of Bengal, Central Indian Ocean Basin, Pacific Ocean and World Oceans

Element	Arabian Sea (LR)	Bay of Bengal*	Indian Ocean#	Pacific Ocean+	World average@
Mn	35.83	24.10	35.02	19.81	16.18
Fe	1.82	1.10	1.38	10.97	6.47
Cu	0.04	0.01	0.16	0.12	0.30
Ni	0.09	0.08	1.44	0.42	0.48
Zn	0.02	0.15	0.37	0.11	–
Co	0.02	0.01	0.16	0.12	0.30
Mn/Fe	4.76–80	10.55–25.42	5–101	1.95–5.37	–

*Chauhan *et al.*[12]; #Pattan[2]; +Stoffers *et al.*[7]; @Poppe *et al.*[21].

below to oxic condition above is thus indicated by the dark brown pelagites in the present case. It is, therefore, inferred that micronodules of the study area have formed under suboxic conditions with diagenetic mobilization of Mn from the deeper levels and its precipitation at upper levels in the sediment column. This is in contrast to the oxic environment suggested for the formation of micronodules of CIB and for some occurrences in Bay of Bengal[12,2,11]. As in the present case, a few sub-bottom occurrences in the latter, however, have formed under suboxic condition too[11].

The sedimentation rate in the Arabian Abyssal Plain is 2–3 cm/10^3 yr (ref. 18). Higher rates of 4.6 to 9.8 cm/10^3 yr recorded from the northern Arabian Sea[19] are probably due to the influence of Indus discharge. The waning influence of Indus influx in the south might be another factor responsible for the abundance of micronodules in the southern part and their near-absence in the abyssal plain further west. Another critical feature is that on the western side the abyssal plain had received maximum turbiditic discharge from the Indus during the last glacial peak and on the eastern side forming the western slope of LR the sediments were contemporaneously laid at very slow rate. This must also have favoured nodule growth in the western slope of the LR but not far west in the abyssal plain.

Bioturbation by benthic organisms is cited as another reason for generating the oxidation processes or the means of Mn and Ni supply to the red clays of south Pacific[20]. In this process the burrowing activity is considered to provide furrows and pellets for Mn coating. Ni enrichment is attributed to precipitation by organic processes. Indications of intense bioturbation are seen in the present cores suggesting that elemental concentration by burrowing mechanism is a distinct possibility.

In conclusion, Mn-micronodules and coatings occur extensively in the slope region, west of the Lakshadweep Ridge at water depths of 2800–4400 m. They are usually found in the 5 to 20 cm thick dark brown pelagic clay host sediment which is interbedded with light coloured inhomogeneous sequence of hemipelagic clay/calcareous ooze. The sediment commonly lies 10–50 cm below the seabed. The abundance of the micronodules in the host sediment varies greatly between 2.6 and 24% by weight of the >0.05 mm fraction, but not exceeding 0.5% of the total sediment.

Birnessite is the dominant mineral in the Mn-micronodules. The nodules are rich in Mn (35.83%) and are in general Fe-depleted (1.82%) with Mn/Fe ratio averaging 31.10. They have highly variable content of common trace metals, viz. Cu, Ni, Co, Pb and Zn. Based on lithological characteristics, chemistry and mineralogy it is inferred that these nodules have been formed under suboxic condition with early diagenetic effect. The nodule growth is enhanced further by low rate of sedimentation and was possibly aided by bioturbation.

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Seasonal variation in infestation characteristics of bagworm (Lepidoptera: Psychidae) in avenue plantation of *Acacia* and *Peltophorum* in the Chhattishgarh region

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Seasonal infestation profile of bagworm, *Eumeta crameri* was constructed based on data recorded over a time scale of twelve consecutive months in the Chhattishgarh region. Two plantation sites consisting of 433 *Acacia nilotica* and 517 *Peltophorum pterocarpum* trees were chosen for the study. Plants were examined at monthly intervals and a visual score assignment system was employed to determine the degree of infestation. Cosinor technique was used to validate statistically significant annual rhythm in infestation characteristics. Results reveal that in both sites maximum outbreak of infestation occurred between November and January in the form of a single peak in each infestation parameter, notwithstanding evidence in favour of two additional generations during the study.

BAGWORMS are a group of highly specialized Lepidopterans belonging to the family Psychidae[1]. There are about 40 species available in the Indian territory[2]. Extreme development of sexual dimorphism in bagworm makes it a biological curiosity. The male bagworm moth is winged whereas the female lacks functional appendages. It spends the most part of its life inside a cylindrical/fusiform bag, which is open at both ends. The bag is constructed by the larva by using silken thread and other extraneous materials, such as leaves, spines, stems and twigs. Until pupation the larva keeps itself on the move from one branch to another in the host plant along with the bag, which is usually referred to as a mobile nest or case.

The bagworm has been documented as a serious defoliator of ornamental shrubs and trees throughout the Southeast and lower Midwest[3]. The potentiality of bagworm as a pest has been studied by several workers[4–7]. In India, until recently bagworms were not recognized as serious pests[2,4]. It has now been clearly established that many species of bagworms have already attained the status of a serious pest in Kerala.

Pteroma plagiophleps (Hampson) causes large scale defoliation of forest plantations of *Albizia falcateria* and *Delonix regia*[8,9]. However, very little is known about the bagworm species prevalent in this country. In this communication we document the prevalence of a population of bagworm species, *Eumeta crameri* and its infestation characteristics in selected avenue plantation sites in the Chhattishgarh region. In addition, seasonal infestation profile of *E. crameri* has been examined.

The *Acacia nilotica* plantations along the NH #6 passing through Bhilai (lat. 20°10′N; long. 81°20′E) and the avenue plantation of *Peltophorum pterocarpum* in the campus of Bhilai Steel Plant were chosen for the study. Plants in both sites with $n = 433$ and $n = 517$ respectively, were examined for bagworm infestation every month for a period of 12 months. A visual score assignment system was employed to determine the degree of infestation[10]. A plant was assigned a score of S_0, S_1, S_2, S_3 or S_4 representing respectively, no, low, medium, high and very high level of infestation. While the low level of infestation was characterized by the presence of at least one bag visible from the ground, the very high level was marked by sizable amount of defoliation[7]. The monthly intensity of infestation was computed and expressed as the ratio of number of plants in each score to the total number of plants, for example $I_1 = S_1/n$ (see Table 1).

Analysis of variance was used to validate statistically significant seasonal effect. In addition, an annual rhyth-

Table 1. Monthly infestation profile of bagworm in the avenue plantations of *Acacia nilotica* and *Peltophorum pterocarpum*

	Infestation intensity				
Month	I_1	I_2	I_3	I_4	I_t
Acacia nilotica					
September	0.127	0.362	0.434	0.016	0.939
October	0.099	0.385	0.457	0.009	0.95
November	0.131	0.401	0.434	0.004	0.97
December	0.097	0.420	0.436	0.006	0.959
January	0.078	0.411	0.454	0.006	1.651
February	0.212	0.413	0.281	0.027	0.933
March	0.295	0.376	0.226	0.050	0.947
April	0.314	0.258	0.217	0.080	0.869
May	0.143	0.210	0.154	0.205	0.712
June	0.110	0.189	0.124	0.374	0.797
July	0.212	0.235	0.256	0.041	0.744
August	0.272	0.212	0.184	0.000	0.668
Peltophorum pterocarpum					
September	0.265	0.228	0.166	0.003	0.612
October	0.245	0.216	0.156	0.003	0.62
November	0.235	0.208	0.116	0.003	0.562
December	0.218	0.162	0.148	0.002	0.53
January	0.294	0.236	0.199	0.003	0.732
February	0.247	0.286	0.156	0.007	0.696
March	0.274	0.267	0.19	0.013	0.744
April	0.197	0.224	0.185	0.029	0.635
May	0.158	0.189	0.168	0.073	0.588
June	0.114	0.156	0.133	0.241	0.644
July	0.208	0.170	0.147	0.013	0.538
August	0.181	0.156	0.119	0.002	0.458

$I_1 = S_1/n$ (ratio of the frequency of plants which were assigned S_1 and total number of plants in the site); $I_2 = S_2/n$; $I_3 = S_3/n$; $I_4 = S_4/n$; $I_t = I_1 + I_2 + I_3 + I_4$.

mic variation for all the levels of infestation was characterized for both sites by the parameters of the best fitting cosine function approximating all data[11]. The rhythm parameters estimated by this least squares method include the mesor (M, rhythm adjusted annual mean), the amplitude (A, half of the difference between minimum and maximum of the best fitting cosine function) and the acrophase (ϕ, time of the annual peak obtained from this cosine function with local midnight of December 31 as ϕ reference). A rhythm is detected, with regard to the considered τ ($\tau \equiv 365.25$ days) when the amplitude differs from zero (non-null amplitude test) with $P \leq 0.05$. This single cosinor method[12] was employed for the original time series and also for the series obtained after fitting 3-item moving average. The latter was employed in order to smoothen the annual curve in the intensity of infestation[13]. Other conventional statistical techniques were also followed whenever required[14].

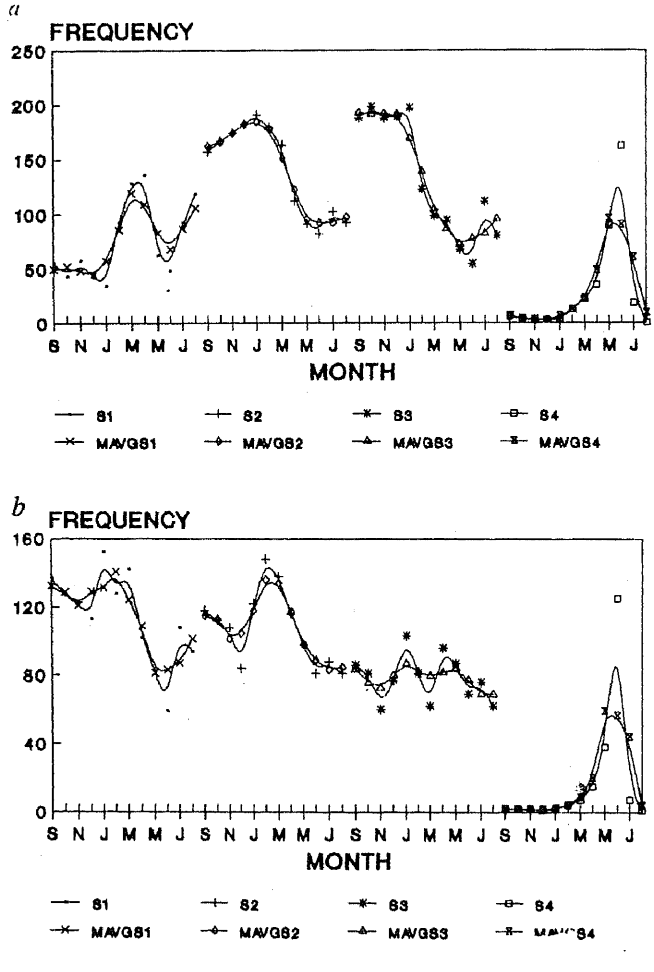

Figure 1. Seasonal variation in the levels of bagworm infestation in plantation sites of (*a*) *Acacia nilotica* and (*b*) *Peltophorum pterocarpum*. S_1, S_2, S_3 and S_4 denote original data indicating low, medium, high and very high level of infestation, respectively. $MAVGS_1$, $MAVGS_2$, $MAVGS_3$ and $MAVGS_4$ denote 3-item moving average fitted data of the time series, S_1, S_2, S_3 and S_4 respectively.

The seasonal variation in intensity of infestation was examined over a period of twelve months. Both original and 3-item moving average fitted time series were plotted as a function of the time of the year (Figures 1 *a,b*). The monthly intensity of infestation was gauged by computing the ratio of number of plants in each score to the total number of plants. The sum of all the ratios in each month was computed and expressed as the total infestation intensity (Table 1). A statistically significant month effect was validated by ANOVA only for I_4 level of infestation intensity (Table 2). The masking of month effect for other intensity of infestation levels could be because of the disparity in infestations between *P. pterocarpum* and *A. nilotica* leading to neutralization of such effect. However, a highly statistically significant species effect was documented for all the levels of infestation intensity excluding I_1 (Table 2). The results reveal that *A. nilotica* was more heavily infested with bagworms than *P. pterocarpum*. Table 3 shows the results of cosinor analysis employed on 3-item moving average fitted time series. A statistically significant annual rhythm was validated for almost all the infestation parameter, excepting S_3 in *P. pterocarpum*. The annual peaks for $S_1 + S_2 + S_3$ infestation intensity occurred on November 27 and December 27 in *Acacia* and *Peltophorum* plantations respectively (Table 3). The peak map is shown in Figure 2, which indicates that 95% confidence limits in peaks for most of the infestation parameters, irrespective of host plants, overlap with each other.

This is the first study that concerns bagworm infestation in the Chhattishgarh region. As such there are limited centers in India where work of comparable nature is being carried out. Prominent among these is the Kerala Forest Research Institute at Peechi. Until 1981 the bagworm, *Pteroma plagiophleps* was known as a minor pest of *Tamarindus indica* in India, when Nair *et al.*[8] demonstrated that *P. plagiophleps* has already assumed

Table 2. Summary of the results of ANOVA* employed to analyse seasonal infestation intensity

Infestation characteristics**	Main effect	
	Species (df 1.11)	Month (df 11.11)
I_1	0.067^{NS}	1.6^{NS}
I_2	26.333^{c}	2.667^{NS}
I_3	15.444^{b}	0.889^{NS}
I_4	7.0^{a}	16.0^{c}
I_t	25.869^{c}	2.087^{NS}

*ANOVA based on data shown in Table 1. Since only one score was obtained for each level of infestation in each month, species × months interaction mean square has been used as the error mean square.
**Computed as the ratio of number of plants in a given level of infestation and total number of plants.
NS = Not significant; $^{a}P \leq 0.05$; $^{b}P \leq 0.01$; $^{c}P \leq 0.001$.

Table 3. Cosinor summary showing annual rhythm in infestation intensity in *Acacia nilotica* and *Peltophorum pterocarpum*. Rhythmometry is based on 3-item moving average fitted data shown in Figure 1

Key[1]	*P*[2]	Mesor ± SE[3]	Amplitude (95% CL)[4]	Acrophase (95% CL)[5]
Acacia nilotica				
S_1	0.003	75.0 ± 5.6	26.1 (02.8, 49.5)	Apr. 18 (Feb. 15, July 21)
S_2	< 0.001	141.3 ± 3.3	50.4 (36.8, 64.0)	Dec. 06 (Nov. 21, Dec 21)
S_3	< 0.001	132.7 ± 4.7	66.7 (47.5, 86.0)	Nov. 06 (Oct. 21, Nov.24)
S_4	0.001	29.9 ± 5.3	41.1 (19.3, 63.0)	May 12 (Apr. 09, Jun. 12)
$S_1 + S_2$	0.005	216.3 ± 6.0	37.6 (12.7, 62.6)	Jan. 06 (Nov. 24, Feb. 18)
$S_1 + S_2 + S_3$	< 0.001	349.1 ± 7.5	92.0 (61.0, 123.1)	Nov. 27 (Nov. 07, Dec. 18)
$S_2 + S_3$	< 0.001	274.0 ± 7.7	113.6 (81.9, 145.3)	Nov. 18 (Nov. 03, Dec. 06)
Peltophorum pterocarpum				
S_1	0.001	113.9 ± 3.3	24.8 (11.0, 38.5)	Dec. 03 (Nov. 03, Jan. 09)
S_2	0.021	107.8 ± 3.6	18.3 (03.6, 33.0)	Jan. 18 (Nov. 27, Mar. 12)
S_3	0.106	78.5 ± 1.4	05.0	Feb. 06
S_4	0.005	17.3 ± 4.0	25.5 (08.9, 42.1)	May 18 (Apr. 09, Jun. 27)
$S_1 + S_2$	0.007	221.7 ± 6.7	39.9 (12.1, 67.8)	Dec. 24 (Nov. 18, Feb. 06)
$S_1 + S_2 + S_3$	0.007	300.2 ± 7.4	43.7 (13.3, 74.1)	Dec. 27 (Nov. 15, Feb. 12)
$S_2 + S_3$	0.013	186.3 ± 4.3	23.1 (05.3, 40.9)	Jan. 24 (Nov. 27, Mar. 12)

[1] Infestation level (S_0 = nil; S_1 = low; S_2 = medium; S_3 = high; S_4 = very high).
[2] From *F* test of null amplitude rejection hypothesis.
[3] Rhythm adjusted mean of best-fitting cosine function ± 1 standard error.
[4] Half of the difference between maximum and minimum of best-fitting cosine function with 95% confidence limit.
[5] Time of maximum (in month and days) in best-fitting cosine function with 95% confidence limit.

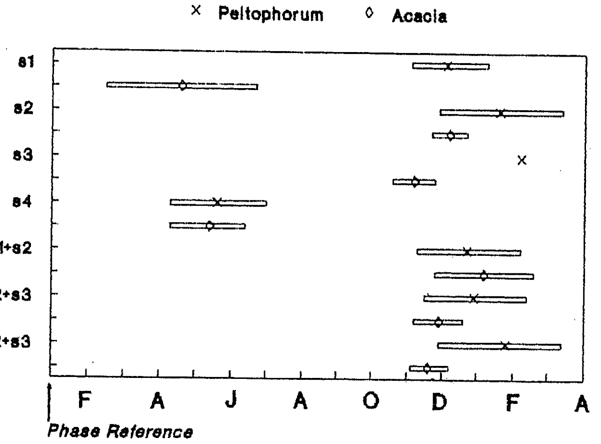

Figure 2. Peak map. Each point represents the peak in the levels of infestation over an annual time scale with December 31 as the phase reference. Both ends of the horizontal bar define the range of the 95% confidence limit of the peak infestation. The absence of bar indicates nondetection of statistically significant annual rhythm.

the status of a serious pest of *A. falcataria*, a fast growing tree species introduced from Mohccas[15]. This suggests that the bagworms are notoriously polyphagus and have the capability to switch over to a new host. It can cause heavy defoliation in various plantation sites. For *P. plagiophleps*, Nair *et al.*[8] reported that it is likely to become a limiting factor for successful raising of *A. falcataria* plantations in Kerala. In addition, *Eumeta crameri*, *Metisa palma* and *Brachycyttarus subteralbatus*, each of them alone or together, have been documented as pests of important tree species, such as *Delonix regia*, *Tectona grandis*, *Manatha albipes*, *Terminalia catappa*, *T. indica* and *Casuarina equisetifolia*.

The studies on the dynamics of seasonal infestation caused by *E. crameri* of avenue plantations of *P. pterocarpum* and *A. nilotica* reveal that in both cases maximum outbreak is restricted to the winter months. The maximum infestation of *Peltophorum* is in December with a spread between beginning of November and mid February. However, in *Acacia* the highest infestation was noticed in November with a spread between beginning of November and the third quarter of December. Although these observations suggest that the bagworms reproduce only once in a year, the actual observations on life cycle of *E. crameri* indicate that it breeds at least 3 times in a year notably in July, October and January. It is not known why 3-item moving average fitted time series did not reveal all the generations in the form of peaks in infestation intensity. Similar kind of masking has also been reported for infestation of *P. plagiophleps* in *A. falcataria* plantations[7]. It could be reasonable to imagine that there might be some unknown natural limiting factors behind this phenomenon of masking. The pest management could become effective only when the principles which govern outbreaks of infestation are known.

This study assumes a lot of significance from regional context specially because it documents infestation profile in *A. nilotica* plantations. This plant is a common sight in the rice fields of the Chhattishgarh and being a leguminous plant it fixes atmospheric nitrogen and its compound leaves with small pinna do not obstruct the sun rays. This plant also has great economic value.

It is being used as fuel, charcoal, timber for agricultural implements and also carpentry[16]. The farmers of this region use its timber in making cart wheel and plough. In addition, it is used to make pulp in paper and rayon industries and its bark in fermentation technology for manufacturing country liquor[17]. It has also been shown to be of great value in the management of rain water in alkali soils[18].

In conclusion, the bagworm appears to be of great economic significance and therefore, a nation wide operation is desirable with a view to documenting the species and their hosts from all the corners of the country.

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Analysis of gene expression during embryonic development in mulberry silkworm *Bombyx mori*

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We have developed a method for dechorionation and devitellinization of the silkworm eggs without damage, to facilitate the analysis of gene expression during embryonic development of *Bombyx mori*. Making use of antibodies available from heterologous systems, the spatio-temporal expression patterns of *peroxidase* and *proliferating cell nuclear antigen* have been directly visualized in whole mount embryos at various stages of development without the need for generating transformed lines carrying specific reporter constructs. The *B. mori* system, previously unamenable for such studies, could thus serve as an attractive model for molecular analysis of insect development.

THE attention lavished on *Drosophila melanogaster* as the insect species *par excellence* for the genetic and molecular analysis of development, has eclipsed the earlier investigations on most other insects for a multitude of challenging biological phenomena. The mulberry silkworm, *Bombyx mori* has a genetic legacy comparable to that of *Drosophila*, but most of the efforts on the silkworm were channelled towards the improvement of the race through breeding, due to its economic importance[1,2].

The extended period of embryonic development of *B. mori* (10 days as compared to 24 h in *Drosophila* and 100 h in *Manduca sexta*, another widely studied Lepidopteran insect) provides an advantage to discretely analyse without overlap, the events leading to pattern formation. The early investigations on *B. mori* were confined to the problems of diapause[3] and storage of embryos or towards the elucidation of morphological landmarks by electron microscopy[3–5] and fluorescent vital dyes[6]. Recently the fate mapping of *B. mori* embryo has been carried out following laser irradiation[7]. The investigations on gene expression during early embryonic development of silkworm have lagged behind because the dechorionation and devitellinization of embryos proved to be major hurdles. Besides, the embryonic development could not be examined due to the nonavailability of cell type-specific molecular markers as well as the lack of transgenic methodologies. In such situations the immunocytochemical approaches provide an alternative. Antibodies against gene products can be exploited as tissue- and cell-specific markers to analyse phenotypes in mutant embryos[8–15]. Recent studies[8–17] have implicated the evolutionary conservation, based on sequence similarity of developmentally important genes, in a range of distantly related species. As a consequence, immunologic probes from heterologous sources could prove to be useful in detailed tracking of individual gene products during development. There is a distinct need, however, to adopt the existing methodologies to the *B. mori* system, where a major hurdle exists due to the presence of a thick chorion layer encasing the egg which affects the permeability of the embryo to external probes.

Here, we have improvised a protocol for the dechorionation and devitellinization of *B. mori* embryos to facilitate the analysis of spatio-temporal expression of genes during development. We have also exploited the heterologous antibodies against *Drosophila* gene products to examine the pattern of gene expression during embryonic development of silkworm and demonstrate that the system is now amenable for such analysis.

B. mori egg is a flattened sphere, 1.2 mm long, 0.95 mm wide and 0.63 mm thick and is encased in an envelope of chorion (Figure 1 *a*). The antero-posterior and dorso-ventral axes of the egg are clearly discernible from the flattened shape of the anterior side. The surface of the chorion shows hexagonal spots with no subdivisions and an anteriorly situated micropyle (Figure 1 *b*) which permits sperm entry.

B. mori follows a characteristic embryonic development pattern which essentially resembles the 'long germband' insects but with distinctive characters of its own (Figure 2). In brief, the major landmarks during embryonic development are syncitial blastoderm (12 h after egg laying), germband formation (20 h), gastrulation (25 h), generation of segmental body plan (35 h) and blastokinesis which initiates from the 4th day and lasts till 5th day. During blastokinesis, the developing larva makes a complete turnaround from its initial position and along with it the developing legs and the pseudopods get located into the interior. This revolution of embryo is an interesting feature of *B. mori* development. By the 7th day the complete functional larva is formed which hatches out only on the 10th day. Studies on the developmental expression of the genes during embryogenesis in *B. mori* could not be done previously due to problems of dechorionation and devitellinization of the eggs. The thick chorion can neither be removed mechanically during earlier stages of embryonic development as in *Drosophila*[18] nor its permeability be increased to stain the embryo without removal of chorion as practised in zebra fish[19]. Moreover, the thickness of the *B. mori* embryos reduces microscopic resolution of the staining patterns.

Eggs (embryos) were washed in distilled water to remove the glue covering them and suspended in PBT (PBS + 0.1% TritonX-100) containing 0.1% proteinase K for 15 min. They were dechorionated in 5% hypochlorite bleach for 5 min followed by the addition of an equal volume of heptane. On dechorionation the embryos pop into the organic heptane layer preventing any damage. We tried different concentrations of the hypochlorite bleach and found that a higher concentration (5%) was necessary to dechorionate *B. mori* eggs. Pretreatment of eggs with proteinase K further improved the effectiveness of the bleach. The presence of heptane allowed the immediate relocation of the dechorionated

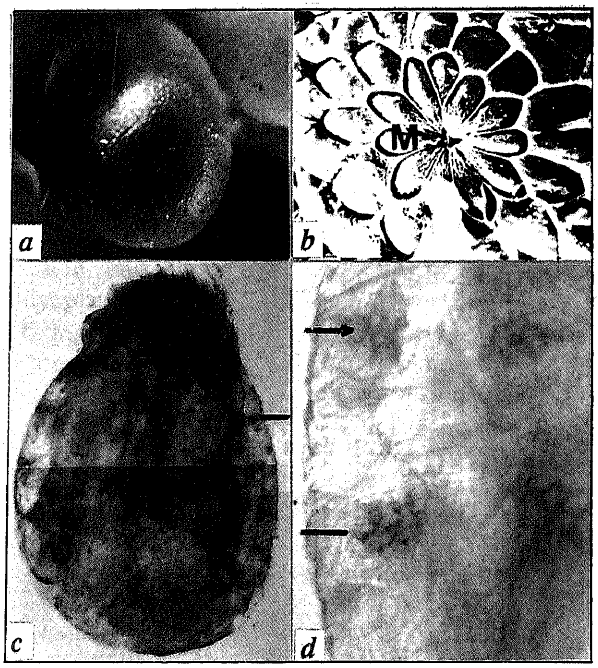

Figure 1. Whole mount embryonic staining using anti-HRP antibody. ***a***, *Bombyx mori* egg (× 15), seen under stereo microscope (Olympus). ***b***, Scanning electron micrograph (JEOL) of the egg shell surface (× 1000).

Eggs were collected at 30 min intervals and maintained at 25°C. The time of embryonic development is referred to as 'hours after egg laying' (AEL) and hatching occurs 10 days after oviposition. For staining, the dechorionated embryos were fixed in equal volumes of heptane and the fixative (4% paraformaldehyde in PBT, 1 mM $MgSO_4$, 2 mM EGTA) for 40 min with continuous shaking. These embryos were flushed thrice with methanol to remove the fixative and stored in methanol at – 20°C. Prior to antibody staining, the embryos were rehydrated in several changes of PBT followed by three washes of PBTA (PBT + 1% bovine serum albumin) for 2 h and incubated overnight at 4°C in primary antibody. After primary antibody reactions, the samples were washed in three changes of PBTA for 10 min each at room temperature, treated with the secondary antibody for 2 h at 4°C and the expression was monitored.

For detecting *peroxidase* expression (as marker for the neuronal development), the dechorionated embryos were treated with anti-horse radish peroxidase (HRP) antibody, followed by treatment with the secondary, goat anti-rabbit IgG coupled to HRP as the detection system. HRP activity was detected by staining with 0.5 mg/ml of diaminobenzidine and 0.01% H_2O_2. The stained specimen were observed under DIC optics on an Olympus microscope. ***c***, Whole mount embryo (6th day of embryogenesis). ***d***, Magnified view of a bundle of sensory bristle on embryonic ectoderm (× 100). Controls, in which the primary antibody was left out prior to the reaction with the anti-antibody, did not show colour development. For comparison of the embryonic development stage, see Figure 2.

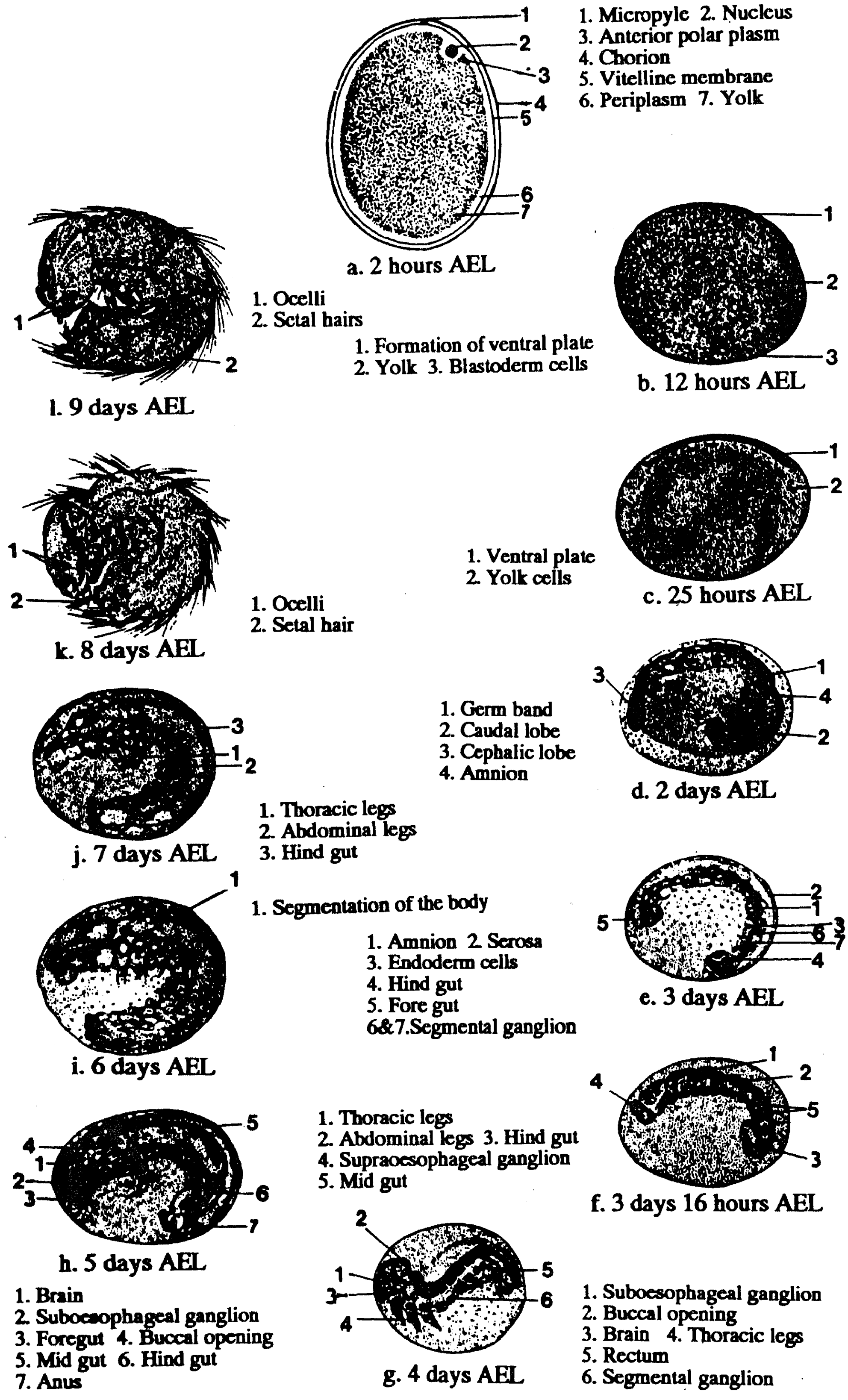

Figure 2. Schematic presentation of the embryonic development of silkworm *B. mori*. Various landmarks of the embryonic development along the temporal scale are depicted. ***a***, Structure of silkworm egg. ***b***, Cleavage and blastoderm formation. ***c–f***, Germband formation. ***g–h***, Blastokinesis. ***i–l***, Completion of embryonic development.

embryos to the organic layer. The relatively brief exposure of embryos to higher concentration of bleach along with an interface of heptane has allowed us to achieve chemical dechorionation successfully with higher yields and less morphological damages.

Such dechorionated embryos were devitellinized in heptane–methanol interphase[18] and examined for the expression of different genetic markers by making use of heterologous antibodies. The expression patterns of *peroxidase* and *proliferating cell nuclear antigen* (*pcna*) were examined using rabbit polyclonal antibodies raised against horse radish peroxidase (HRP) or *Drosophila* PCNA respectively. The appearance of peroxidase activity has been taken as an expression marker for nervous tissue development[20]. The expression of *peroxidase* was detected in basal cells of the sensory hair on the ectoderm of the silkworm embryo at the 6th day of development (Figure 1 *c,d*; arrowhead). Suitable controls were included while conducting the antibody staining reaction (where the samples were treated only with the secondary antibody with no prior treatment of primary antibodies) to rule out any possible artefacts. In fact, little information has been documented in the past on neuronal development during silkworm embryogenesis. The present approach thus serves as a convenient means for such analyses, if various nervous system specific markers are used as probes along the temporal scale.

The approach for the detection of gene expression was extended further to study the expression of *pcna*. PCNA is involved in several vital processes of cell division and DNA replication, and its presence could be expected in all dividing cells during development. Using antibodies, *pcna* expression was detected initially at 10 h of development at the cellular blastoderm stage confined to the periphery of the embryo (Figure 3 *a*). At 20 h of development *pcna* expression further evolved in the developing germband on the ventral side of the embryos, marking all the developing segments (Figure 3 *b*). During gastrulation movements at 25 h after egg laying, it was expressed in all the developing segments of the silkworm embryos which further refined at the end of 35 h expressing in all the seven head-, three thoracic- and eleven abdominal segments of the larval body plan (Figure 3 *c,d*).

Using the methodologies established here, the *B. mori* system with its excellent genetic backup can be exploited to serve as an alternative model system to study gene expression. The extensively studied insect *Drosophila*

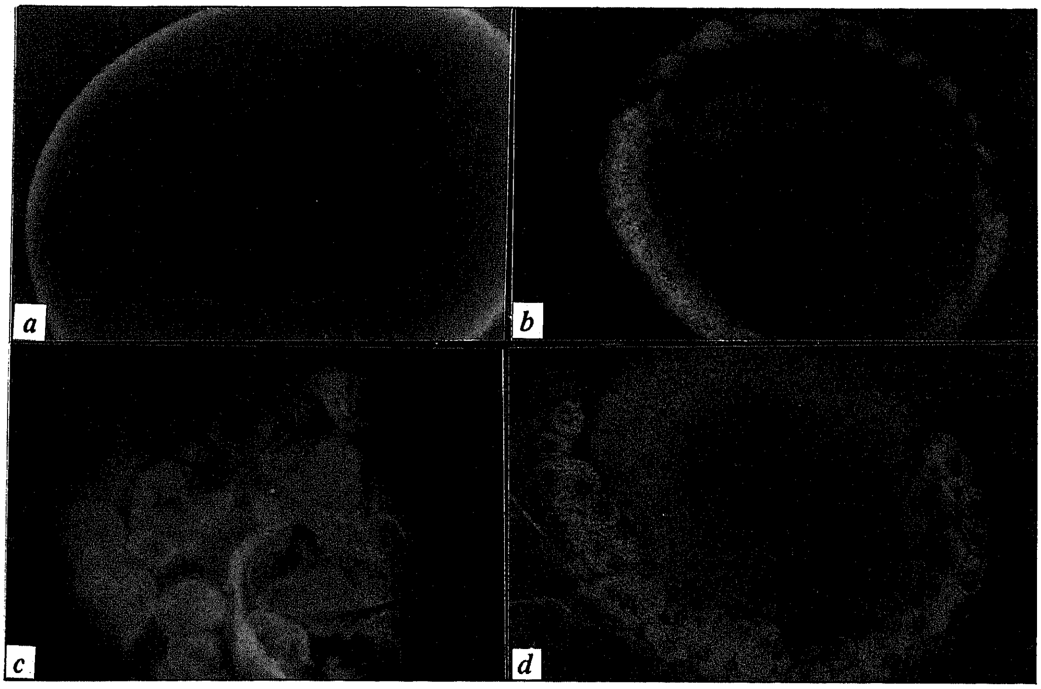

Figure 3. Embryonic expression of *proliferating cell nuclear antigen*. The dechorionated embryos were treated with rabbit polyclonal antibody to *Drosophila* PCNA and the reactions were visualized using goat anti-rabbit antibody conjugated to fluorescein isothiocyanate. The specimen was examined under an Argon Laser Confocal Microscope (Biorad MRC 1000). ***a***, Expression at the syncitial blastoderm stage, optical cross section at 10 h after egg laying (AEL) (× 10). ***b***, Developing germband with formation of the segments as shown by arrow head at 20 h AEL. ***c***, Cellular movement of gastrulation at 25 h AEL. ***d***, Embryo at 35 h AEL with well-developed germband. Note the expression is uniformly distributed in the entire germband with several pockets of rapidly dividing cells. Controls in which the primary antibody treatment was omitted, were always included, and they did not show staining. For comparison of the embryonic development stages, see Figure 2.

belongs to the phylogenetically evolved order Diptera and not necessarily represent all insect groups. The embryogenesis in *Drosophila* is rather unusual compared to other Arthropods[21–24]. Comparison with the more 'typical' insects may provide further insights as to which aspects of embryogenesis are widespread and which are phylogenetically restricted. Lepidopterans being separated by more than 200 million years from Diptera, should provide an important window on evolutionary innovations relative to *Drosophila*. This will offer two complementary approaches deducing both ontogenic and phylogenetic mechanisms[23].

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Annual Review of Medicine: Selected Topics in Clinical Sciences. Coggins, Hancock and Levitt, eds. Annual Reviews Inc., 4139, El Caminoway, P.O. Box 10139, Palo Alto, California 94303–0139, USA. Vol. 47, 1996, 596 pp. Price: US $ 52. Elsewhere $ 57.

The Annual Review of Medicine is different from others such as the *Annual Review of Biochemistry* in that the subject matter is broad, covering every aspect of laboratory and clinical medicine. At least in this sense the *Annual Review of Clinical Medicine* is a misfit in the stable of superbly focused publications produced yearly by Annual Reviews Inc. This is not to detract from the quality of effort that has gone into assembling the contents. Indeed, with 44 articles crowding 566 pages this may be the most densely packed of the Annual Reviews, reflecting an attempt to be eclectic as well as comprehensive in the limited space available.

Given burgeoning research in human molecular biology, it is not surprising that molecular medicine is well represented in the contents. Many of these articles offer tantalizing glimpses into the future of medicine and it may be many years before these ideas are translated into practical applications. However, innovations in medicine are not always byproducts of molecular biology. Some important advances relate to better treatments, as well as better use of existing treatments. Lastly, it should be kept in mind that modern medicine is extremely expensive and the 'good old' remedies still have a place, provided their role is reassessed continuously, in view of the rapid flow of new information. The articles in this volume pay heed to all these themes and a few examples will serve to illustrate this.

While it is well recognized that the 'placebo effect' is a real and measurable response of patients to inert principles no effort has been made to systematically harness it for the benefit of patients. Indeed, physicians are trained during the evaluation of new medicines to devise ingenious means, such as masking, to discount the placebo component of a patient's response to medicines. The article on the Placebo Effect by Herbert Benson and Richard Friedman makes a case for 'harnessing' the power of the placebo to benefit patients in the practice of clinical medicine. Since the response to an active medicine will also have a placebo component it is difficult to understand what the authors mean when they exhort the reader to 'incorporate it into standard treatment'. However, the authors do make the valid point that the 'placebo' component of response can be maximized through closer and increased doctor–patient interactions. No doubt, proponents of alternative systems of medicine will take issue with the authors' suggestion that most alternative medicines are placebos. Some may also question the need to replace the elegant and well-understood term 'placebo effect' with the clumsy neologism 'remembered wellness'. Medicine is full of meaningless jargon without our having to add more.

Gene therapy finds place in this volume in the form of a review by Cynthia Dunbar of the NIH, USA, of the use of hematopoietic cells as vehicles for introduction of genes. Hematopoietic cells are conveniently accessible and the progeny derived from the original stem cell also carry the introduced gene, making available a clonal population of cells producing the gene product. This technique is advanced enough to have reached early clinical trials. The results from these studies and the development of new vectors for the transfer of genes will place gene therapy on a firm footing. Also falling within the domain of molecular medicine are excellent reviews on 'trinucleotide instability', X-linked severe combined immuno-deficiency', 'the p53 gene' and 'apolipoprotein E alleles in Alzheimer's disease'.

The article on the mechanism of action of lithium in the treatment of manic depression is a catalogue of results from experiments that aim to prove that the action of lithium in modulating brain function is a result of depletion of inositol. While the findings are provocative they are far from being conclusive and most clinicians would probably not find this contribution of any practical interest. There are several rather confusing findings that the authors gloss over. One glaring finding is that while lithium decreases brain inositol, inositol itself has antidepressant effects. The hand waving resorted to at the end of this chapter does not resolve this paradox in a satisfactory manner.

There are two articles that diabetologists may find interesting. One covers 'beta-cell function' and the other discusses the 'genetics of NIDDM'. Although insulin resistance is the current fashionable topic among diabetologists, the former article reminds us of the importance of beta-cell dysfunction in NIDDM characterized by an abnormal insulin response to a glucose load. No doubt, the controversy regarding which came first, insulin resistance or beta-cell dysfunction, will remain with us. The latter article on the 'genetics of NIDDM' is probably fascinating to researchers who have many potential targets to fish for but dissatisfying to clinicians who are not presented with the possibility of an integrated molecular approach to NIDDM. Research in this area is still in 'the more we understand the less we know realm'. In a similar vein articles on 'myocardial preconditioning for cardioprotection' and the 'role of antiinflammatory drugs in Alzheimer's diseases' offer a fascinating glimpse into what may become possible in the near future.

A few chapters seem a little out of place in this collection such as the one on the 'implantable cardioverter defibrillator', 'laparoscopic cholecystectomy', 'laxative abuse', 'fatal asthma' and 'Bartonellosis'. These contributions may be better suited for a textbook of medicine rather than the Annual Reviews. The editors should also possibly consider issuing a separate volume with the title 'Annual Reviews of Molecular Medicine' given the accelerating growth of new information in that area.

A set of up-to-date references accompany each contribution and makes this volume useful for those seeking the original contributions. The compact size of the book makes it suitable for bedside reading. A large number of line drawings and schemes have been included that makes the subject matter easily accessible. However, the labels associated with the figures are of poor quality. The small blurb that appears at the end of each chapter advertising ordering information for reprints is a little irritating and could probably have been made to appear once at the end of the book.

The very reason that this compilation is unique may be why this book may lack punch. Within the covers of this book are reviews spanning such a variety of topics that only the generalist with plenty of time will be able to read more than a minor fraction of the articles. One gets the feeling that unlike subjects like biochemistry, the topics that fall under the rubric of clinical medicine are not amenable to coverage in one volume without a persistent undercurrent of tension resulting from the need to provide both breadth and depth of coverage. Nevertheless, for the rare (at least in India) breed of laboratory clinicians there is enough in this volume to make it a delightful companion.

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Mineral Resources of Karnataka. B. P. Radhakrishna, Geological Society of India, P. B. No. 1922, Bangalore 560 019. 471 pp. Price: Rs 200, $ 25.

Mineral Resources of Karnataka by B. P. Radhakrishna (BPR, as he is fondly referred to within the earthscience fraternity) represents the handiwork of the right-man-on-the-right-job. None perhaps qualifies better for this specific job.

The 471-page volume, written with a view to 'disseminating available know ledge and educating the public on the mineral resource potential of the State', is divided into two major sections. The first part (though not explicitly labelled as such) comprising four chapters deals with history of two different kinds: the developmental *history of the mineral industry* in Karnataka since ancient times, set against the backdrop of the Indian mineral–industrial scenario; and the *tectonic-metallogenic evolutionary history* of the region in the context of global metallogenesis in space–time coordinates. BPR is eminently knowledgeable about and authoritatively competent in handling both kinds. The second part comprises 'commodity reports' on various types of mineral resources of the State.

The entire text, especially the preface and the introductory section, reflects the author's economic philosophy that has a Gandhian aura about it: self-sufficiency, self-reliance, small-scale labour-intensive mineral enterprises in rural areas; emphasis on development of low unit-value commodities (like sand or building stones) that are useful to virtually everyone; repeated plea for indigenous technology and for export of 'processed' goods rather than 'raw' ore materials, etc. A conviction, that the small-is-beautiful concept is *appropriate* and *applicable* in our context, pervades throughout. One may disagree and consider all this too simplistic in an era when giant multinational mining corporations rule the roost the world over, but the conviction unmistakably stems from his personal, and successful, experience, with the Hutti–Tintini–Chitradurga tie-up in which he had been a major influence.

A few statements are slightly disconcerting though. Like 'Non-ferrous metals . . . although not abundant, can still meet the limited *requirements of the State*' or '. . . mineral resources have not been exploited to the *best advantage of the State and its people*' (p. 2), or 'Mineral development and administration are *best left to the individual States* (p.v. – all italics mine). Surely no 'myopia' needs be casually diagnosed, no sub-nationalism needs be read here – not from BPR, a national figure in his prefession. But such emphasis may send wrong signals that the interest of the country and that of its constituent states are not compatible.

Since the targetted readership is 'the public' (presumably including legislators, planners, administrators and other decision-makers) as also the earth scientists, the balancing job has proved to be rather daunting. The reviewer, for one, was excited at the prospect of sharing the rich experience and wisdom of BPR but had to be content with summarized treatments of geology of the ore deposits – a treatment dictated by the choice of 'the public' as the primary target for whom simplification is often inescapable. Nevertheless the contention, that 'ocean floor . . . volcanic rocks spread out and become continents through successive collision and separation' (p. 30), is *unacceptably oversimplified.* Similarly, placing the stratiform complexes (with Cr, Ni, Ti, V mineralization) under 'Volcanogenic mineralization' (p. 44), or classifying arenite-hosted copper sulfide deposits as 'detrital or placer deposits' (p.161) would have few takers!

Some discrepancies in the hierarchy of sub-heading should better have been avoided; for instance, under the heading 'ARCHEAN' (p. 35–40), subheads like 'Gold in banded iron formation', 'Gold in Greywacke', 'Au–U bearing quartz pebble conglomerate', etc. enjoy the same hierarchical status – anomalously, to my mind – as those on the 'Eastern Schist Belt' and the 'Western Schist Belt' both of which deserve higher-order status in the scheme of organization.

The section on 'Commodity Reports' (p. 47–471), arguably the *raison d'etre* for the book, bears the unmistakable stamp of BPR's intimate knowledge of virtually every single deposit/occurrence in Karnataka. Geology of important deposits is discussed – briefly, simply and lucidly for the general readers, but alas somewhat inadequately for professional geologists. The section could perhaps have done with some standard formatization (like that in the Mineral Commodity Summaries published by the US Bureau of Mines). Was the inclusion of items like 'andalusite' warranted really?

The production is trim and attractive, virtually free from printing errors. Reproduction of geological maps and cross-sections is of high quality as also are the beautiful colour photographs of polished ornamental stones.

Overall, the book is a valuable addition to our rather meagre repository of texts on geology and economics of mineral deposits of the country or of a State. If the review sounds captious at any point, it is because expectation from BPR is always high. How much I wish the text were to discuss the geology of ore deposits in Karnataka in sufficient detail and present the compilation of mineral statistics and mine directory as an appendix!

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Fundamentals of Aquacultural Engineering. Thomas B. Lawson. Chapman & Hall, USA. ISBN 0-412-06511-8. 355 pp. $ 45.

With the global fish production fast approaching the maximum sustainable yield, the role of aquaculture as an important contributor to high quality food production has become more apparent in recent years. The concept of aquaculture has shifted from traditional methods to more of a commercial operation, with attempts to maximize the production from a given waterbody. Commercial aquaculture is not simply a biological operation, but a complex, multidisciplinary activity involving the expertise of biologists, engineers, economists and others. In this context, the *Fundamentals of Aquacultural Engineering,* stands out prominently among the volumes of books on aquaculture. The author, basically a freshwater aquaculture engineer has brought out the fundamentals of aquaculture engineering principles in simple technical terms in the 12 chapters of this book.

Aquaculture production, to a great extent, is influenced by the physical, chemical and biological characteristics of the environment and their interactions. The aquaculturist should have know ledge of water quality and the environmental requirements of the cultured organism for the venture to be successful. Chapter 2 provides practical information on water quality standards for aquaculture. The illustrations and examples are mostly drawn from freshwater aquaculture and may not be directly applicable to mariculture though the basic processes are common to both the systems. Chapters 3 and 4 briefly highlight the factors that should be considered while selecting a suitable site and water source for freshwater aquaculture.

Among the various culture systems developed for open waterbodies, the technology for cage culture has evolved essentially from engineering techniques. The bulk of the section on aquaculture in open systems presents a fairly good overview of this subject. Inadequate knowledge of water requirements and poor selection of pumps can result in significantly increased operating and maintenance costs and result in crop failure. In the three chapters, fluid mechanics, pumps and flow estimation and measurement, the author presents the basic engineering principles of fluid dynamics, functioning of different types of pumps and methods for sensing and maintaining water levels in ponds with illustrations and practical examples. This information will be very useful to the aquaculture engineer as well as the entrepreneur.

Success of aquaculture in ponds, raceways and tanks to a great extent depends on the engineering design of the system. Chapter 9 provides the basic structural designs and tips for water and waste management in these three advanced culture systems. This information which rarely finds place in many of the aquaculture books is quite useful for the aquaculturists. Good quality water is likely to become a limiting factor for aquaculture, and therefore, water reuse systems have been gaining more attention, recently. Recirculation systems offer advantage of water saving, reduced risk of external contamination and better environmental control. The author deserves appreciation for a detailed presentation concerning the principles and various methods of water treatment in the chapter on Recirculating Aquaculture Systems. Equally commendable is the chapter on oxygen and aeration which discusses the fundamental principles of aeration, aerator devices and application practices in aquaculture. In fact, the author earmarked a major portion of the book to this important aspect as many farmers are ignorant of the benefits of aeration on production. The last chapter on sterilization and disinfection is very relevant as disease transmission is on the increase with the intensification of aquaculture. There are a number of readily accepted methods of treatment. The merits and demerits of different treatments are broadly discussed.

This book is undoubtedly an useful practical guide not only to the aquaculture engineering students, but also the fishery biologists, the practising engineers and the aquaculturists. Most of the examples are from temperate freshwater aquaculture, though aqua- culture is more of a tropical occupation. The book deals basically with freshwater aquaculture engineering, but it is equally useful to the mariculturists. The exhaustive bibliography will be beneficial to the students in obtaining additional information on specific topics of interest in their respective fields.

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Pramatha Nath Bose (1855–1934)

B. P. Radhakrishna

Three years ago, I was pleasantly surprised to receive a letter from Dr Chandan Roychaudhuri, Secretary of the Asiatic Society, conveying the decision of the Council of the Society to award the Pramatha Nath Bose Memorial Medal to me for the year 1990. Excepting a vague recollection that P. N. Bose was connected with the discovery of the rich iron ore deposits of Gurumahisani, I knew little about him for his contributions to knowledge. My curiosity to know more about this first Indian to join the Geological Survey of India was aroused and since then I have been able to collect some information about the life and achievements of this remarkable personality. I would like to share this knowledge with the readers of the journal so that they too can come to recognize the merits of this great son of India and his contributions to the advancement of earth science education in this country.

Early life

Pramatha Nath Bose was born on 12th May, 1855, at Gaipur, a village then in the district of Nadia, but now in 24-Paraganas of Bengal, the second child of Taraprasanna Bose and Sashimukhi. Recalling his boyhood days at Gaipur, Pramatha Nath has written 'we had the five best physicians – sunshine, air, water, exercise and diet'.

Pramatha Nath had his early schooling in the *Bangabidyalaya*, the village school, till he was nine years old and then moved to Krishnagar where he came under the influence of the famous Bramho reformer Keshab Chander Sen. He conceived the idea of going to England for higher studies and appeared for the competitive examination to secure the coveted Gilchrist Scholarship instituted in 1887–88 for talented Indian students. He stood first in the examination and secured the scholarship which was tenable for five years.

Stay in England

Pramatha Nath sailed for England in September 1874 and stayed in London for nearly six years. The subjects which he chose for study included chemistry, botany, geology, zoology, physical geography and logic. After his graduation from the London University in 1877, securing the third position in geology, he joined the Royal School of Mines for a short period. He took part in political meetings and started criticizing the acts of government. Officials of the India Office did not like his activities and wanted to pack him off to India. It is said that the British Government of the day went out of the way to offer him a job in the Geological Survey of India. Bose has recorded 'This was the first case in which the Secretary of State exercised his discretion in favour of an Indian in regard to the appointment in his patronage'.

Marriage with Kamala

Pramatha Nath returned to India in July 1880, a true nationalist and on July 24, 1882, married Kamala, daughter of Ramesh Chander Dutt, a noted Sanskrit scholar. It is interesting to recall that the marriage was attended by many literary celebrities of the day including Bankim Chandra, the author of *Vande Mataram* and the budding poet Rabindranath Tagore. Rabindranath has recalled in his autobiography (*Jeevan Smruti*) that when Ramesh Chander Dutt came to garland Bankim Chandra, the great novelist took the garland from Dutt's hand and placed it round the neck of the young poet Rabindranath much to the astonishment of those present. Rabindranath later became a pupil of Bose.

Service in the Geological Survey of India

Pramatha Nath joined the Geological Survey of India on 30th July, 1880, i.e. earlier than La Touche (1881) and Middlemiss (1883). He was the first Indian to join the Survey on a graded post and served the Survey for 23 years. The short history of the first hundred years of the Geological Survey records that Bose was the first to introduce into the Survey, the study of micro-sections as an aid to petrological work and the first to give descriptions of micro-sections in Progress Reports. During the period of his service, he carried out geological surveys of the Narmada Valley, of Rewa State, Central India, in the districts of Balaghat, Raipur, Mandla and Bastar, and on the Shillong plateau. He also reported on the manganese deposits of Jabbalpur district, coal in Darjeeling district and copper in Sikkim.

Bose was the first one to record the occurrence of trachyte from the Narmada region. In his classical memoir of 1883, he reported the presence of Lameta and Bagh beds and used them as marker beds for correlating similar sequences in other parts of India. He was the first to identify distinct volcanic centres around Mandaleswar in the Narmada Valley.

He took leave for two years between 15th May, 1895, and 15th May, 1897, mainly to write and see through the press his monumental work *A History of Hindu Civilization under British Rule* published in three beautifully bound volumes. Much later (in 1921) he wrote another book *National Education and Modern Progress*.

Reprinted from *J. Geol. Soc. India*, 1997, **49**, 1–5.

Resignation from the Survey

In normal circumstance, Pramatha Nath Bose, being the seniormost among the officers had to be promoted as Director of the Survey. However this did not happen. Bose has recorded – 'Indians are required to run a race with one of the swiftest peoples of the modern world on paths hitherto unknown to them but familiar to their foreign competitors'. The British government of the day was reluctant to put an Indian at the head of the prestigious Geological Survey. It held the view that it was suicidal for the Europeans to admit that natives can do anything better than themselves and that they should claim to be superior in everything and only allow natives to take a secondary or subordinate post. Holding such a view the Government of India, instead of promoting Bose appointed Thomas Holland, who had joined the Survey much later, as the new Director. Bose in protest tendered his resignation and retired from service on 1 December 1903, when he was only 48 years and still had seven more years of service. This was a bold step to take during those days and required great deal of moral courage.

Service to the iron and steel industry

The loss to the Survey was a gain to the Mayurbhanj State which immediately appointed Bose as State Geologist. This gave an opportunity to him to examine in greater detail the iron ore occurrences which he had spotted in his earlier surveys. Convinced of the richness and extent of the deposit at Gurumahisani and other nearby places in Singhbhum district, he got in touch with J. N. Tata, the great industrialist and was able to persuade him to shift the site of the steel plant which he intended to locate near Dalli–Rajhara to Sakchi (present Jamshedpur). One would have normally taken full credit for so great a discovery but not so Bose. His modesty made him say that he was only instrumental in bringing to light what had long been known to the indigenous smelters. Bose negotiated with the Maharaja of Mayurbhanj State and secured for the Tata's, a mining lease over an extent of 20 sq. miles on favourable terms. Bose had a prophetic vision and firmly believed that the firm of Tata's would before long come to be reckoned as one of the great steel producers of the world.

Bose was thus instrumental in ushering in and nurturing the iron and steel industry in India. In recognition of the services rendered to the house of Tatas, a bust of Pramatha Nath Bose was erected at Jamshedpur which was unveiled by Lewis Fermor, Director of the Geological Survey of India on 13th March, 1938.

Bose was also in the forefront of the swadeshi movement which rocked Bengal in the early years of the 20th century. He was instrumental is starting the Bengal Technical Institute in July 1906, and functioned as its Honorary Principal. A princely legacy left by Rash Behari Ghosh, enabled the Institute to shift its location to Jadavpur near Calcutta and the name was changed to the College of Engineering and Technology, Jadavpur, in 1929 and later became the Jadavpur University. Bose advocated imparting technical education based on western methods but adapted to our national needs. In his rectoral address (1914) he said

> 'We talk loudly of national progress but are oblivious of the elementary principles of such progress that a nation has to work out its own salvation by its own effort, and that the more help we take beyond a certain limit the more helpless we become.'

These words have great relevance today when intensive efforts are being made to borrow money and know-how from other countries. There can be no substitute for hard work and self-reliance.

A perusal of Bose's private letters to members of his family reveal his emotional and affectionate qualities surcharged with strong national spirit. He emphasized again and again the importance of plain living and high thinking, activated by a self-sacrificing missionary zeal, not only in religious practices, but also in social, educational and industrial fields. He was convinced about the superiority of Indian culture and exhorted his countrymen to cultivate it assiduously and not blindly copy the customs and mannerisms of the West. He deprecated the sense of inferiority which affected his countrymen and strongly urged them to feel proud of their own past.

Ranchi Ashram

Pramath Nath purchased 21 bighas of land in Ranchi in 1907 and started building a large house. He settled down to farming and the Ranchi house became an attraction to his artistic friends. His house, reverberated with music and song and the merry prattle of children and grandchildren, especially during the Pooja holidays.

In his retirement at Ranchi, Bose kept himself active, communicating his views on various matters of current interest regularly to the noted periodicals of that period – *The Modern Review of Calcutta, The Indian Review of Madras, The Calcutta Review, Prabuddha Bharatha and the Amrit Bazar Patrika.*

He was not particularly enamoured of the achievements made by the West in Science and Technology. He felt that this had only helped the promotion of militarism and forged fresh fetters on the weaker peoples. In all the development he witnessed around him he found much science no doubt, but hardly any consideration for human welfare. There was much learning but wisdom was lacking. He pleaded for rescuing humanity from the morass of militarism, malevolence, greed, selfishness and vice. His biographer, Jogesh Chandra Bagal, states that 'Service of the Motherland was the mission of his life and his pen was wielded in her cause to the last'.

Last days

Pramatha Nath enjoyed good health. He had not much faith in western medicine and never consulted a doctor. He was hopeful of completing his eightieth year and looked forward to having a grand celebration on that day. However, this was not to be. After a brief illness he passed away on the 27th April, 1934, at the ripe age of 79. The last rites were performed on the banks of the Subarnarekha river. *Amrit Bazar Patrika*, mourning his death wrote:

> '. . . at one time he was a Pucca Saheb and blind admirer of everything western. But gradually the awakening came and the Pucca Saheb became an Indian in the truest sense of the term. It was a maxim with him that if India has to attain real freedom, she must follow the great culture evolved by the *Rishis* of old and what he believed, he not only

preached and translated into practice himself.'

Connection with the Asiatic Society

The association of Pramatha Nath with the Asiatic Society extended as far back as 1883. Taking an active part in the affairs of the Society for over fifty years he wrote a monograph surveying the development of scientific research in India during the hundred year period between 1784 and 1884.

The Society instituted a Pramatha Nath Bose Memorial Medal for recognizing conspicuously important contributions to geology, with special reference to Asia. The award is triennial, the first award being made in 1950. Many eminent geologists including West, Auden, Krishnan, L. Rama Rao, Pichamuthu and Gansser have been the recipients of this award in earlier years.

'Let us pay tribute to this pioneer scientist and geologist of India, from whose work so much good has resulted.' In these words Pandit Nehru, the first Prime Minister of India, praised Pramatha Nath Bose and his services to the country. Let us not forget our early benefactors.

B. P. Radhakrishna is in the Geological Society of India, P. B. No. 1922, Bangalore 560 019, India.

PHYSICAL RESEARCH LABORATORY
AHMEDABAD 380 009

The Physical Research Laboratory makes four awards called 'Shri Hari Om Ashram Prerit Dr Vikram Sarabhai Research Awards', every two years from funds kindly provided by Pujya Shri Mota of Hari Om Ashram of Nadiad. These awards will be made to Indian scientists, who are below 45 years of age, on 1 January 1997 for original work in the following fields:

1. Space Sciences (including Astronomy, Astrophysics, Planetary and Atmospheric Sciences).
2. Space Applications (in the areas of Meteorology, Hydrology, Remote Sensing and related ground truths).
3. Electronics, Informatics, Telematics and Automation.
4. Systems Analysis or Management including non-linear, non-equilibrium systems in natural and social sciences and technology.

Although the overall work of the candidates would be taken into account, the work done in India would be given prime consideration.
The candidate should have to his credit at least one or more of the following achievements:

1. Significant achievement in scientific research.
2. Important and successful adaptation of new technology.
3. Planning, development and implementation of systems in the context of science and technology.

The selection for the year 1997 will be completed by January 1998 and the awards presented on 12 August 1998.
The last date for receiving nominations is **15 September 1997.** Sponsors are requested to send a two-page note (12 copies) summarizing the contributions and achievements of the sponsored candidate together with his/her biodata, complete list of publications (with the names of the authors in the order in which they appear in the publications), reprints of five most important publications during the last five years (6 copies) in a cover marked 'confidential', addressed to the **Director, Physical Research Laboratory, Navrangpura, Ahmedabad 380 009.**
More detailed information will be asked for by the Selection Committee, if considered necessary.

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Opinion articles present views on issues related to science and scientific activity. **Commentary** articles should contain expository notes on issues related to science and scientific activity.

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1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355–362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A.), Academic Press, New York, 1970, vol. 1, pp. 319–322.

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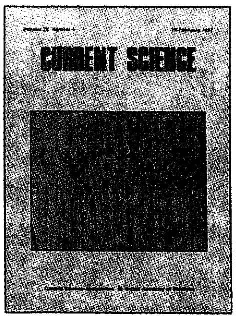

COVER. Longitudinal section of *Millettioxylon indicum* Awasthi showing storied arrangement of xylem rays. See page 248.

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In this issue

How physicists burn their fingers

'Does steam cause a more severe burn than boiling water?' asks M. D. Deshpande (**page 235**). The chorus, from generations of teachers and students of basic physics, brainwashed by the magic words 'latent heat' would be a resounding yes. Our author's first task is to show that this standard examination question has as many holes in it as the proverbial sieve. He then draws on the accumulated wisdom of fluid dynamics (Prandtl, Reynolds, Nusselt,...) to come up with and answer well-posed versions (the plural is significant) of the same question. And lo and behold, water causes the more severe burn in all the cases. But the goal is no mere one-upmanship over the pedants. One emerges with a new respect for real world problems and their refusal to fit into simplistic academic categories. A physicist would be additionally chastened by the sharp observation that his laboratory is a wilder, more uncontrolled place than the chemist's!

R. Nityananda

Longevity and overcrowding: The case of the fruit fly

Ageing is inevitable and mortality is an inescapable fact of life. Understanding the factors that determine lifespan is the central concern of gerontologists. A grand unified view of ageing appears to be emerging as evolutionary biologists develop 'an approach that combines demography, population genetics and physiology'. Joshi and Mueller (**page 255**) use the fruit fly *Drosophila melanogaster* to address the effects of adult overcrowding on longevity. Their title points to the main conclusion – that there is an increase in age-independent mortality. This should surely strike a chord in societies struggling with enhanced population densities. The experimental protocol and statistical analysis provide clues as to the advantages of choosing the fruit fly as the subject of investigation. The authors report results which should excite the curiosity of even non-biologist readers. For instance, female flies appeared to be affected by overcrowding to a greater extent than males, showing dramatically diminished longevity. Is discrimination based on sex an important feature? The authors hasten to add 'unfortunately too much cannot be made of these differences because the early death of large numbers of females in the crowded treatment would tend to increase life span in males due to reduced courtship and mating'. The authors conclude that 'adult crowding affects longevity by altering the age-independent mortality rate rather than the rate of ageing'. For females there is the trade-off between 'fecundity and longevity'. What is the underlying physiology that is responsible for the observed effects of overcrowding? Both depletion of lipid reserves and glycogen have been considered in other studies. A more elaborate picture will emerge only when physiological and biochemical analyses of the effects of overcrowding are undertaken.

P. Balaram

Current Science

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We expect that electronic submission will result in quicker processing for publication.

CURRENT SCIENCE

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CORRESPONDENCE

Monitoring India's lifescape

I was pleased to read the article 'Deploying student power to monitor India's lifescape', by Madhav Gadgil (*Curr. Sci.*, 1996, **71**, 688–697) and the painting on the cover page. The painting by Sanjeeva Nayaka was done extremely well. However, I noticed that Nayaka, for some reason or other, has missed out an important flying mammal of the order Chiroptera. These fascinating mammals known as 'bats' have existed even before pre-homosapien era. Inclusion of these creatures would have really completed the scenario on the cover page.

RIKI KRISHNAN

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Bangalore 560 012, India

Madhav Gadgil replies:

I fully share Krishnan's affection for the bats. In fact I enjoy nothing more than watching the swarms of giant fruit bats flying over the campus of the Indian Institute of Science every evening. However, I am also fond of bugs and beetles, wolves and whales. Unfortunately it is not possible to accommodate all these creatures in a depiction such as was attempted for the cover of the November 10, 1996 issue. Therefore, we had to be content with selecting just one organism to represent each phylum. An exception was made in the case of two major phyla, namely Arthropoda and Chordata. For these we have representatives of the major classes as well. For mammals we chose a land animal – a deer, since the greater diversity of mammals prefers to walk rather than fly or swim. It was this logic that forced us to leave out bats. I hope that Krishnan would appreciate our dilemma and accept our apologies on behalf of the bats.

MADHAV GADGIL

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Extramural research funding in India – An analysis for 1994–95

Of the 1557 R&D projects approved for funding to the tune of Rs 13133.76 lakhs by 16 Central Government departments/agencies during 1994–95, Bangalore-based Indian Institute of Science tops the tally with 64 R&D projects worth Rs 873.12 lakhs. Among the top 10 beneficiaries, Delhi alone has four such organizations (Table 1) which justifies its status as the R&D capital of India (*Curr. Sci.*, 1993, **65**, 114–115). These data are contained in the latest directory of extramural projects brought out by the Department of Science and Technology (DST).

Specifically, R&D projects in the disciplines of biological (25.43%) and engineering (24.73%) sciences together account for slightly over half of the total amount. Among various categories of institutions, the universities/colleges (233) received 57.23% of the approved projects. Interestingly, only 420 organizations and 14 individual scientists shared these 1557 projects of 3–62 months' duration.

In so far as the funding profile is concerned, the DST ranks first amongst the 16 participating agencies (Table 2) both in terms of number of approved projects (458) and their total cost (Rs 4559.38 lakhs). Of these 1557 R&D projects, 721 received grants ranging between Rs 1 and 5 lakhs and only 70 projects figure in the category of Rs 25 lakhs and above. Of the four megaprojects costing

Table 1. Top 10 organizations with number of projects and total approved cost during 1994–95

Ranking	Organization	Total approved cost in Rs lakhs	No. of R&D project(s)
1.	Indian Institute of Science, Bangalore	873.12	64
2.	OPTEL Telecommunications Ltd., Bhopal	407.50	1
3.	University of Delhi, Delhi	360.21	32
4.	Indian Institute of Technology (IIT), Delhi	348.23	36
5.	Indian Institute of Petroleum, Dehra Dun	346.61	2
6.	IIT, Mumbai	329.40	44
7.	Jawaharlal Nehru University, New Delhi	293.35	25
8.	All India Institute of Medical Sciences, New Delhi	258.06	32
9.	IIT, Kanpur	245.08	34
10.	Centre for Cellular and Molecular Biology, Hyderabad	238.69	6

Table 2. Central Government departments/agencies which furnished the list of projects approved during 1994–95

Name of the agency
Council of Scientific and Industrial Research
Defence Research and Development Organization
Department of Atomic Energy
Department of Biotechnology
Department of Coal
Department of Education
Department of Electronics
Department of Ocean Development
Department of Science and Technology
Indian Council of Agricultural Research
Indian Council of Medical Research
Indian Meteorological Department
Indian Space Research Organization
Ministry of Water Resources
Ministry of Welfare
University Grants Commission

Rs 100 lakhs and above, the Department of Biotechnology sanctioned two whereas DST and the Department of Electronics sanctioned one each.

While discussing its limitations, the directory puts on record that the Ministry of Non-Conventional Energy Sources and Ministry of Environment and Forests did not furnish the list of projects for this endeavour. This is really sad. Hopefully, with the cooperation of all concerned, the future edition will provide a complete picture. It is high time that DST brought out a series of publications highlighting the research output of extramural projects to mark its silver jubilee (1971–96).

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Dengue epidemic

The report on dengue hemorrhagic fever (DHF) epidemic in Delhi and the threat of yellow fever by V. P. Sharma (*Curr. Sci.*, 1997, **72**, 10 and 1996, **71**, 948) should be an eye opener to the Health Ministry and related governmental agencies. It certainly appears that this fatal viral disease (DHF) has come to stay and is likely to come back with more devastating consequences in the coming years. One logical reason for this will be as follows a) all 4 serotypes of dengue (types 1, 2, 3 and 4) have been involved in the previous 6 outbreaks in Delhi; b) population previously infected with any one serotype of dengue does not suffer from serious consequences, but when infected later with a different serotype, most likely suffers from the more fatal form of DHF which could lead to dengue shock syndrome (DSS). This was explained to be due to antibody-dependent enhancement (ADE) of viral infection (review by Halstead, *Science*, 1988, **239**, 476) which is known to occur in a number of flaviviruses to which dengue belongs. Hence a classical vaccine does not appear to be an attractive answer to control DHF. Instead, preventive measures for growth of *Aedes aegypti* mosquito are certainly the best immediate measures to control this disease. We need to learn lessons from the previous epidemics of dengue elsewhere. In Cuba, dengue epidemic occurred for the first time in 1977–78 in which 40% of the total population was infected with serotype 1 but a subsequent epidemic in 1981 due to type 2 virus resulted in DHF in which 1% of the total population (116,000) was hospitalized over a three-month period (Kouri *et al.*, *Bull. Pan Am. Health Org.*, 1986, **20**, 24). Our country is definitely not geared to take up such an emergency involving even a fraction of percentage of population. In the Cuban epidemic, children were the most affected but in the Delhi epidemic even adults were affected which warns us about the changing pattern of this disease. Apart from controlling the vector, there is an urgent need for a brainstorming session involving a cross section of expert scientists who need to act in a coordinated fashion. Instead, only a few individuals and institutes are working on shoe-string budget to battle this gigantic problem. We need to build a national resource of pathogenic organisms which need to be isolated periodically from endemic areas and epidemics and a comparative evaluation of the virus should be undertaken at the molecular level. Unlike the plague disease which at least can be cured by tetracylines, this deadly viral disease has no such drugs. Until a clear national policy is evolved for controlling epidemics, we will be helping history to repeat itself.

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Organometallic chemistry of C_{60} beyond η^2-C=C co-ordination: Buckminsterfullerenes as pentahapto (η^5) cyclopentadienyl ligands

Ramaswamy Murugavel

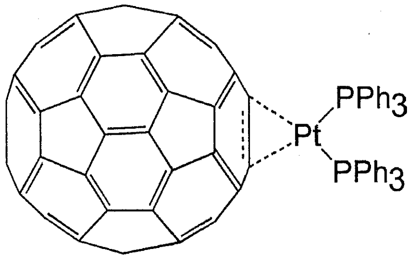
Since the initial discovery and the subsequent development of efficient synthesis of fullerenes[1], studies on the interaction of these molecules with metals have received considerable attention. This is in view of the fact that the metal-containing fullerene derivatives have the potential for the realization of new high surface area catalysts and hybrid materials with exceptional properties. Compared to the well-known chemistry of endohedral fullerenes of the type $M@C_{60}$ (ref. 2), there have been only sporadic reports in the last few years on the chemistry of exohedral organometallic complexes of fullerenes[3,4]. The initial work of Fagan and co-workers in early nineties on the exohedral organometallic chemistry of fullerenes revealed that the C_{60} molecule behaves more like an electron-deficient alkene such as tetracyanoethylene rather than relatively electron-rich species such as ethylene or benzene[3]. Moreover, the short/long bond alteration found in the six-membered rings of C_{60} suggested that each of the double bonds is somewhat electronically localized, resulting in relatively low ring currents in C_{60}. Due to these now obvious reasons, the fullerene co-ordination chemistry has been dominated by only one mode of ligation[3,4], viz. an η^2-coordination through the C=C double bonds which are located at the junction of two adjacent six-membered rings (the 6–6 bonds) as shown in $(\eta^2\text{-}C_{60})Pt(PPh_3)_2$ (A) (ref. 3).

In an important breakthrough in fullerene organometallic chemistry, Sawamura *et al.*[5] have recently described the first successful synthesis of pentahapto (η^5) co-ordination complexes of C_{60} (**2–5**) with alkali main group, and transition metals by elegantly modifying the C_{60} skeleton in terms of its electronic properties (Scheme 1).

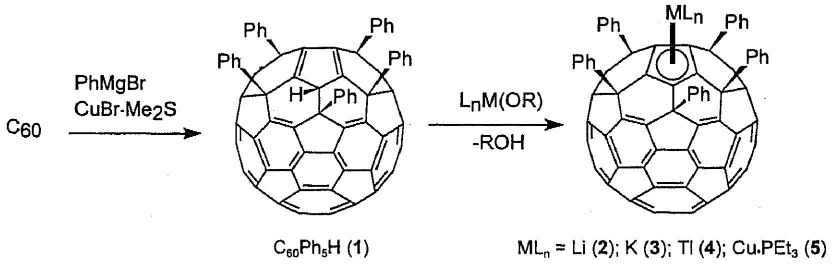
Before going into the details of the recent findings of Sawamura *et al.*[5], a brief introduction to the η^5-cyclopentadienyl organometallic complexes is desirable. Since the serendipitous discovery of ferrocene by Kealy and Pauson[6], the organometallic chemistry of transition metals and many lanthanides has been virtually dominated by the use of cyclopentadienyl (Cp) or substituted cyclopentadienyl (Cp′) ligands which commonly co-ordinate to the metal in a pentahapto (η^5) fashion through all the five ring carbon atoms[7]. One of the main and notable reasons for the vast expansion of Cp-organometallic chemistry over the decades has been the ability of these ligands to support a wide range of metals also tolerating their various oxidation states. Moreover, the ability of the appropriately substituted Cp ligands to dictate desired electronic and steric properties on various metals has led to the preparation of a multitude of catalytically useful systems (e.g. the classical olefin polymerization catalyst Cp_2TiCl_2/Et_2AlCl) (ref. 8).

In spite of the parallel advances both in (a) the η^5-Cp-organometallic chemistry of transition metals and lanthanides and (b) the π-complex chemistry of fullerenes, until the report of Sawamura *et al.*[5], there have been no major attempts to combine these two aspects and devise routes to hitherto unknown η^5-C_{60} organometallic complexes. The main reasons for the lack of synthetic routes for η^5-C_{60} complexes can be easily discerned from the above stated findings of Fagan *et al.*[3]. The fact that the C=C double bonds of C_{60} are localized has so far precluded any possibility of using these molecules as polyhapto ligands.

In complexes **2–5**, the five cyclopentadienyl type carbon atoms are represented by one of the five-membered rings of C_{60}. This pentagon is electronically isolated from the remaining 50 sp^2 carbon atoms of C_{60} by the five surrounded sp^3 carbon atoms, each of which bear an exocyclic phenyl substituent. The key step in the synthesis of complexes **2–5** lies in the successful synthesis of the precursor C_{60} molecule $C_{60}Ph_5H$ (**1**), for which the authors have essentially used the strategy employed by Hirsch *et al.*[9] for the preparation of organodihydrofullerenes. While the



A



Scheme 1.

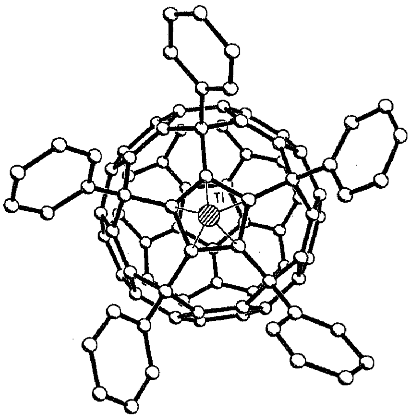


Figure 1. The top view of the molecular structure of $Tl(\eta^5\text{-}C_{60}Ph_5)$. 2.5 THF showing the C_5 symmetry.

reactions of C_{60} studied by Hirsch *et al.*[9] with organometallic reagents such as MeLi or PhMgBr yielded the monoaddition products of C_{60}, the remarkable reaction with an excess amount of the organocopper reagent $Ph_2CuMgBr$ (prepared from PhMgBr and $CuBr\cdot SMe_2$) readily results in the 5-fold addition product $C_{60}Ph_5H$(**1**) as a reddish amorphous solid in 94% yield[5]. It should be noted that, the fullerene **1** has also been prepared by Kroto and coworkers through aromatic electrophilic substitution of benzene with $C_{60}Cl_6$ followed by the reduction of the resulting $C_{60}Ph_5Cl$ in the presence of PPh_3 (ref. 10). However, the later procedure involves a multi-step synthesis which results in somewhat lower isolated yields of **1**.

The reactions of **1** with monovalent metal alkoxides LiO^{t}Bu, KO^{t}Bu, TlOEt and Cu(O^{t}Bu)(PPh_3) yield the respective η^5-$C_{60}Ph_5$ complexes **2–5** (ref. 5). A single crystal X-ray diffraction study of the thallium(I) complex **4** reveals that the molecule possesses an aesthetically pleasing C_5 symmetry with the five Ph groups forming a chiral propeller array as shown in Figure 1. In spite of the chemical structural changes around the Cp ring, the C_{60} core in **4** largely retains its original spherical symmetry. Moreover, the average C–C single and double bond lengths in the C_{50} fragment of the molecule are found to be very similar to those found in monofunctionalized C_{60} derivatives. More interestingly, the electronic spectral data of **1** and **4** reveal that the intensity of the absorption of the 50-π-electron C_{50} chromophore ($\lambda_{max} = 240$ nm, $\varepsilon = 1.1 \times 10^5\ M^{-1}\ cm^{-1}$) is comparable to that of the parent C_{60} suggesting that the electronic properties of the compounds **1–5** are similar to those of C_{60}.

The elegant approach demonstrated by Sawamura *et al.* has truly opened up the door to the exploration of C_{60} coordination chemistry beyond its previously known limits. Carefully planned research on this type of complexes might prove useful in the discovery of a new class of fullerene-based catalysts and novel materials. Among several intriguing challenges in this direction, the preparation of metallocene complexes based on C_{60} (such as ferrocene) and other double/triple decker C_{60} complexes (which would require suitable functionalization at the diagonally opposite ends of the sphere) is likely to draw the immediate attention of the synthetic chemists around the world.

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Research snippets (*Compiled by A. V. Sankaran*)

K–T meteorite relics found

While the enigma about the mass extinction of dinosaurs 65 million years ago (K–T period), whether due to terrestrial or extra-terrestrial agencies is still baffling scientists, that a 10 km wide asteroid or meteorite crashed during that period over Yucatan Peninsula (Mexico), carving the huge Chixulub crater 150 kilometers across, is not being doubted (Figure 1). Though, presumably this meteorite vapourized completely upon impacting, leaving no trace except for their extra-terrestrial signature as iridium enrichment in site-sediments, search for unburnt relics of the meteorite continued. Recently, two teams of researchers exploring for such bits, came across materials which they

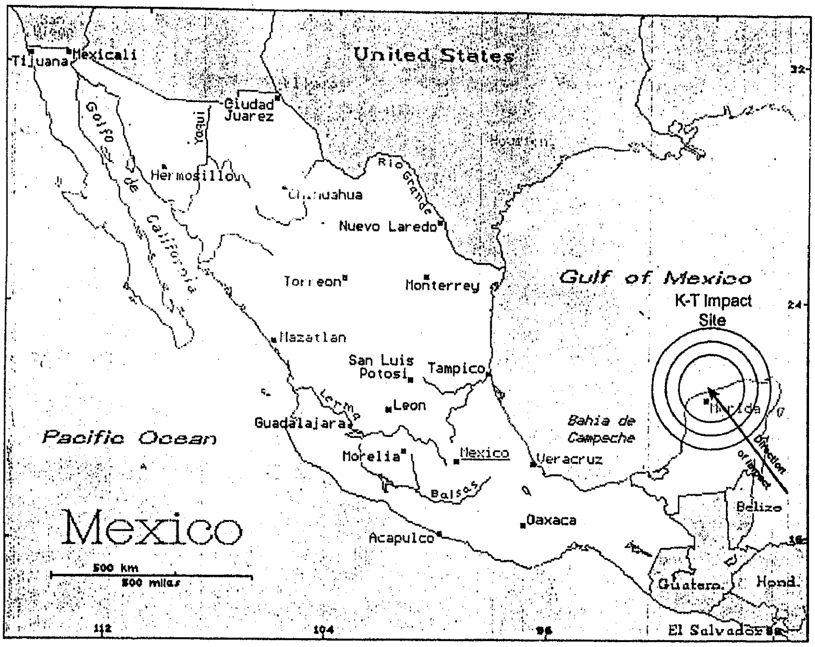

Figure 1. The K–T meteorite impact site (Chixulub Crater) in Yucatan Peninsula, Mexico.

infer were part of this K–T meteorite. One of them[1], Benjamin Schuraytz and colleagues at the NASA's Johnson Space Center, Houston, isolated from the Chixulub crater, the impact site, nuggets of nearly pure iridium enclosed inside a silicate. Apart from this, they also found iridium enriched (13.5 ppb) particles from the impact-generated melt-rock and melt-breccia and the enrichment values happened to be far above those typical of Earth's crust. They suppose that these abnormal amounts of iridium are due to admixture of meteoritic material (chondritic) into the impact melt[1], and they have estimated that at least 3% of chondritic material must have been incorporated.

The second find by Frank Kyte of the University of California, Los Angeles, has come from mid-Pacific, about 9,000 km due west of Yucatan impact site, from drill-core samples retrieved by a team drilling the ocean bed. The meteorite relic they found was a 5 mm long inclusion in a core of dark-brown clay lying at the bottom of K–T boundary layer. This inclusion contained a 2.5 mm long pebble having high amounts of Cr, Fe and Ir, in quantities characteristic of meteorites. They infer that the most reasonable source of this material, undoubtedly deposited within the K–T layers, must belong to the K–T projectile itself. This view is strengthened by the fact that this mid-Pacific site lies exactly in the path of debris that must have been thrown in a northwesterly direction when the meteorite hit Yucatan from the southeast. The impact is believed to have been made at a low angle of 20° to 30° which is considered most destructive.

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A planet rotating inside Earth's womb

Seismological and computer simulation studies about the internal structure and composition of Earth, in an effort to understand the temporal variation of its magnetic field and reversal of its poles, have brought out one of its most interesting aspect – an independent spinning of the inner core, a region about the size of the moon deep in the womb of Earth.

The structure of earth into lithosphere, asthenosphere, transition zone, lower mantle and core has been largely inferred through seismic wave studies. These waves (chiefly the P and S waves, so designated on the basis of their vibration modes) travel through the Earth with different velocities, and they are affected by the density and elastic constants of the materials through which they pass. Thus, they get deflected at surfaces of discontinuity and as a result distinct subzones have been recognized within the mantle and core regions; for example, the core is made up of an outer zone about 2270 kms thick, of molten iron mixed with lighter elements and an inner one of solid iron with a radius of 1220 kms.

According to Walter Elasser and Edward Bullard, Earth's core is a self-exciting dynamo, generating its own magnetic field[1]. Here the fluid iron is 'stirred into convective motion by heat generated from residual radioactivity in the core' and in the presence of small, stray magnetic field, this motion would produce electric currents (which in turn will create its own magnetic field) just as in a dynamo where a conductor (copper wire or disc) moving through a magnetic field of a bar magnet generates electric currents. Now this phenomenon has triggered two geophysicists, Gary Glatzmaier of Los Alamos National Laboratory in New Mexico and Paul Roberts of the University of California, Los Angeles[2–4], to predict on the basis of computer simulations studies, that

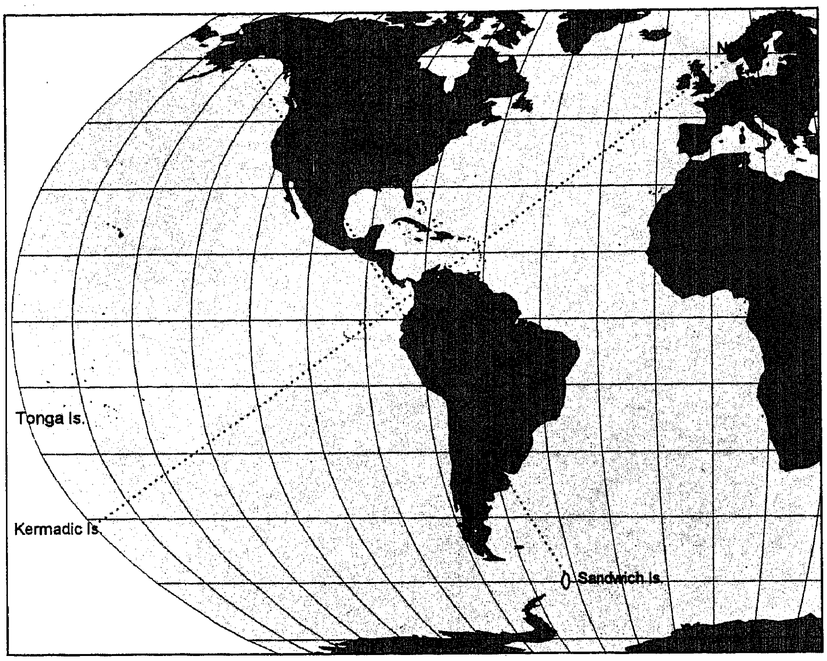

Figure 1. Earthquake focus sites and receiving stations.

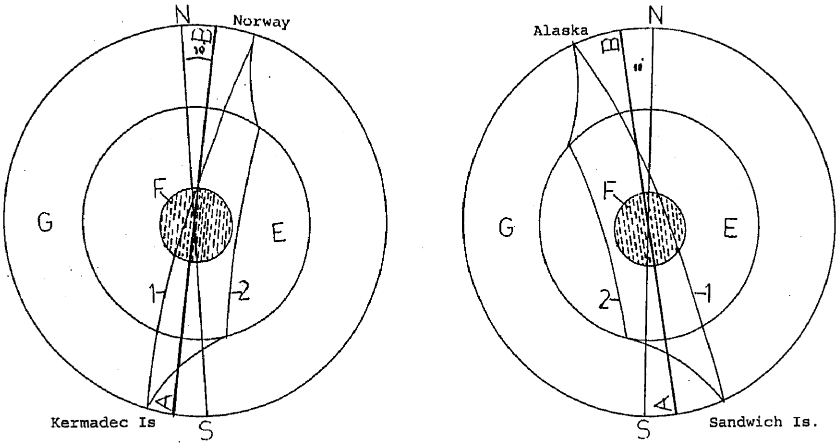

Figure 2. Idealized diagram showing drift of axis of anisotropy due to rotation of the inner core and the passage of seismic waves from earthquake foci at Kermadec and Sandwich Islands in the Southern hemisphere and arriving at the receiving stations in Norway and Alaska in the Northern hemisphere: (*left*) the position of the axis of anisotropy in 1967 and (*right*) its position in 1995. A–B, Axis of anisotropy (fast axis); N–S, North–South axis of Earth; E, outer core; F, inner core; G, mantle; (1) P-wave travelling through both the inner and outer cores, (2) P-wave travelling through the outer core only.

this dynamo action of the inner core of the Earth can induce it to spin freely. They argue that the magnitude of electric currents generated across the boundary with the solid inner core is quite huge (of the order of a billion amperes) and in the presence of magnetic field, this will exert a pulling force on the inner core. Since the outer core is viscous, they calculated that this tug will result in imparting a motion or spin to the inner core by about one or two degrees in an eastward direction.

However, according to another model developed by Arnou, Brito and Olson of Johns Hopkins University, Baltimore, this tug is caused by the powerful electromagnetic field created by stormy vortices of thermal currents raging along a north–south oriented cylindrical zone of relatively hotter outer core fluid, immediately adjoining the inner core[14].

Their predictions about the differential spin of the inner core have now been proved to be true by latest seismic studies by Xiadong Song and Paul Richards of Lamont-Doherty Earth Observatory[5]. Their observations were based on an unique behaviour of the P-wave travelling through the inner core. These waves are known to travel faster inside the inner core in the direction close to the N–S axis but slower along the equatorial plane[6,7]. This is attributed to the preferred N–S orientation exhibited by the high pressure hexagonal polymorph of iron crystals (according to one view[8], the inner core is thought to be one giant single crystal) that make up the solid inner core[9,10]. This direction, better known as the 'fast axis' or the 'axis of anisotropy', is tilted by about 10° to Earth's N–S axis of rotation[11].

Song and Richards examined all the earlier seismic data of earthquakes arising from almost the same site for several years and evaluated the time P-waves took to reach receiving stations located at the opposite sides (antipodes) of Earth (Figure 1) after travelling through (a) both the inner and outer cores (Ray 1, Figure 2) and (b) outer core only (Ray 2, Figure 2). Their examination of these earlier data pertained to the travel times of such pairs of P-waves from (i) 38 earthquakes (> 5.5 magnitude) originating from Sandwich islands (located between Cape Horn and Antartica) in the southern hemisphere and arriving at the receiving station, College, in Alaska (ii) quakes from Tonga island (mid-Pacific), and reaching Graefenberg in Germany, stations lying close to the equatorial plane and (iii) Kermadec island N.E of New Zealand, and arriving in Norway, stations lying close to the N–S axis of anisotropy. They found that while the travel times of the P-waves taking the Tonga–Germany route close to the equatorial plane, remained the same over the years, the ones that took the Sandwich–Alaska route arrived at the receiving stations 0.3 seconds sooner in 1995 than they did in 1967.

Song and Richards argue that since the paths of the P-waves through Earth are essentially fixed, their shorter travel time between Sandwich and Alaska progressively over the 28-year period can take place only if the angle between the paths of P-wave and the fast axis of anisotropy gets gradually reduced. This reduction in the angle is possible only if the inner core rotates, a feature predicted by Glatzmaier and Roberts earlier. The inferences deduced by Song and Richards from past seismic recordings were further strengthened when they noticed that the travel time between Kermadec and Norway, stations which were initially close the fast axis of anisotropy, showed an increase with the passage of time, a clear indication that these two stations are no longer close to this fast axis (Figure 2). Their calculations indicate that the 'inner core rotates about the N–S axis like the rest of the planet, but at a rate that is 1.1° faster', eastwards.

However, subsequent work by Wei-Jia Su and his colleagues from Harvard and University of California, Berkeley, evaluating a 29-year data from International Seismological Centre (1964–1992) has revealed that the inner core rotates about 3° per year faster[12].

The differential spin of the inner core was, in fact, envisaged as early as 1986 and 1989 by the Indian scientist J. J. Rawal, Nehru Planetarium[13], Bombay, as an outcome of the progressive slowing down of the spin of the Earth, a view endorsed later by Raymond Jeanloz, University of California, Berkeley. They felt that this slowing down is due to friction caused by lunar and solar tides and that this deceleration is not fully transmitted to the inner core owing to the existence of a fluid zone of outer core separating it from the rest of the planet. According to Jeanloz, the inner core is rotating presently at a rate Earth's surface was doing some 60,000 to 100,000 years ago.

These findings about the inner core rotation can have great bearing on our understanding of the physics and chemistry of the core, particularly about the outer core viscosity, its temperature and melting conditions, heat flux across the core–mantle boundary, about the dynamo action, geomagnetic reversals and the precession of equinoxes.

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Does steam cause a more severe burn than boiling water?

May be not necessarily. This question is making rounds of our high schools and examinations and has even entered the popular science literature. Often it is posed in such a way that we are not asked to choose whether it is right or wrong but to explain why it is so. The *expected* answer, we know, is that steam causes a more severe burn due to the high latent heat of evaporation.

The question whether steam causes a more severe burn than boiling water is vague and ill-posed and strictly speaking cannot be answered. But what is being done here is to see how far can we rationalize this question and push analysis to understand the related issues. This exercise should also warn us while posing similar *clever* questions.

Steam and boiling water can exist at varying temperatures but it is only correct to assume here that both are at the same temperature. But this aspect is not as simple as it appears. We may have them at the same temperature at the source, but that does not necessarily mean that they will be at the same temperature when they contact the skin to cause a burn. This problem will be discussed later.

Water indeed has an impressively high latent heat of evaporation $H = 2.257 \times 10^6$ J/kg at 100°C and this aspect must have led some science teacher to coin this question. It is quite possible that the question has been

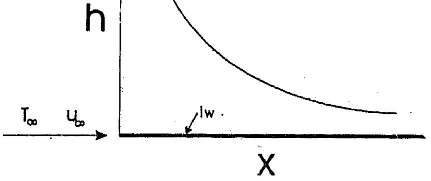

Figure 1. Heat transfer coefficient *h* due to flow along a flat plate.

Table 1. Properties of saturated water and dry steam at 100°C at one atmospheric pressure

Quantity	Water	Steam	Units
Density, ρ	960.63	0.5977	kg/m^3
Specific heat, C_p	4216.1	2076	J/kg°C
Viscosity, μ	2.8×10^{-4}	12.45×10^{-6}	kg/ms
Kinematic viscosity, ν	0.294×10^{-6}	20.83×10^{-6}	m^2/s
Thermal conductivity, k	0.68	0.02408	W/m°C
Prandtl number, Pr	1.74	1.07	–

Enthalpy of evaporation (Latent heat) $H = 2.257 \times 10^6$ J/kg.

Table 2. Non-dimensional variables appearing in this analysis

Reynolds number $Re_x = u_\infty x/\nu$
Reynolds number $Re = v_0 d/\nu$
Prandtl number $Pr = \mu C_p/k$
Nusselt number $Nu = hx/k$ or hd/k

reinvented. Luckily the expected answer has been uniformly the same. But this innocent-looking question fits into a class where only qualitative arguments are used while completely ignoring other important aspects that are involved. The experimental observation is usually ignored or selectively chosen to strengthen the proposition.

The severity of a burn depends on the high temperature to which the skin and the flesh are raised, how long this temperature is held and the area (or volume) of the affected body. The temperature of the affected area will be higher if heat is supplied at a higher rate and also if it diffuses away at a slower rate. If a hot fluid (water or steam) comes in contact with skin, several factors decide how much it affects the skin – the temperature, density, velocity, thermal conductivity, specific heat, latent heat, how much fluid is there and whether it is replenished, how long it is in contact, etc. A droplet of boiling water that falls on the skin quickly loses its heat and cannot cause much damage but a larger volume of it can cause a severe scald. Thermal properties of the skin and the surrounding tissue also play an important role.

Now a quantitative comparison is made for two simple model problems to understand *the interplay of various factors.*

Flow over a flat plate

This simple model is considered for comparison between heat transfer when either water or steam at temperature $T_\infty = 100°C$ flows with velocity u_∞ over a semi-infinite flat plate held at temperature T_w. The distance along the plate x is measured from the leading edge as shown in Figure 1. In the case of human skin $T_w = 34°C$. We assume that the flow remains laminar without becoming turbulent. Even though the steam is bound to condense on the plate, we ignore it at this stage to study the effect of other factors involved.

Heat flux q (in W/m^2) is given by

$$q = h\Delta T,$$

where h is the average heat transfer coefficient and $\Delta T = T_\infty - T_w$ is the temperature difference which may be taken to be 100–34 = 66°C. Reynolds number Re_x (see Table 2) and Prandtl number Pr affect h which is usually expressed by proper scaling in terms of the Nusselt number $Nu = hx/k$. Expressions for Nu for different problems are available in the books on convective heat transfer. From the expression for Nu for the present laminar boundary layer case we obtain

$$h = 0.664\ (k/x)\ Re_x^{1/2}\, Pr^{1/3}.$$

The value of h decreases as one moves along the flow as shown in Figure 1. The singularity at $x = 0$ is integrable and also does not cause any problem for comparison purpose. We use suffix w for water and st for steam. The heat transfer coefficients h_w due to water and h_{st} due to steam can be compared at the same location:

$$(h_w/h_{st}) = (k_w/k_{st})((Re_x)_w/(Re_x)_{st})^{1/2}\ (Pr_w/Pr_{st})^{1/3}.$$

Now k and Pr are fluid properties but Re_x is a flow property since it depends on u_∞ and x also. Then to make a comparison we have to know the values of Re_x in both the cases. We consider the two cases here – same u_∞ or same Re_x.

Case I. Comparison for same u_∞

If u_∞ is the same for the two cases of either water or steam flowing, then Re_x scales like $1/\nu$ leading to

$$(h_w/h_{st}) = (k_w/k_{st})(\nu_{st}/\nu_w)^{1/2}\ (Pr_w/Pr_{st})^{1/3}.$$

Substitution of values for fluid properties from Table 1 gives

$$(h_w/h_{st}) = (28.2)(8.4)(1.18) = 280.$$

This means that heat transfer rate is higher in case of water by a factor of 280. This factor is due to higher conductivity of water (28.2 times), higher kinematic viscosity which is due to higher density (8.4 times) and higher Prandtl number (1.18 times). Of course, we have avoided the question of latent heat for the time being.

Case II. Comparison for same Re_x

We take another case here where Re_x is assumed to be the same in both the cases. This case is selected because of its simplicity. Then

$$(h_w/h_{st}) = (k_w/k_{st})(1)^{1/2}(Pr_w/Pr_{st})^{1/3} = (28.2)\ (1)\ (1.18) = 33.2.$$

Here high k and Pr of water lead to 33.2-fold increase in heat transfer as compared to that due to steam. Note that to maintain the same Re_x, steam has to move much faster – by a factor $(\nu_{st}/\nu_w) = 70.9$. Even then water can burn the skin more severely, if we ignore the latent heat of condensation.

The case of jet impingement

Here we assume that a jet of fluid (boiling water or steam) with velocity v_0 impinges on a flat plate, modelling the skin, as shown in Figure 2. The jet has a diameter d and its exit is at a distance Z from the plate. S is the stagnation point and we compare heat transfer in the neighbourhood of this point. Suffix '0' is assigned to the values at this point. The Nusselt number at this point $Nu_0 = h_0 d/k$. Nu_0 depends on the non-dimensional parameters Re, Pr, Z/d. It also depends on other parameters like turbulence level in the jet, nozzle shape and the boundary conditions at the wall and has the form

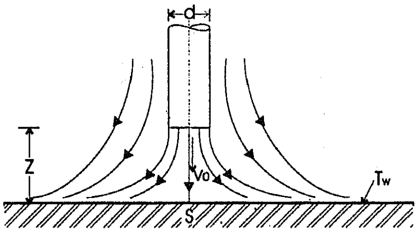

Figure 2. Jet impingement on a wall.

$$Nu_0 = CRe^n Pr^{0.4} (Z/d)^m.$$

An essential difference between the two jets should be mentioned here. The above expression pertains to a fluid jet immersed in the same fluid. Hence it can be a reasonable approximation for steam jet immersed in air or water jet immersed in water but not water jet immersed in air. There are reasons to believe that a water jet in air leads to a higher value of Nu_0 than when immersed in water and hence in what follows Nu_0 will be an underestimate if we take the same expression for water jet in air also. V. Krishnamoorthy has done extensive experiments to quantify Nu and has determined the constants C, n and m as functions of other parameters. I borrow the constants from his results but do not particularly bother to read these constants very precisely, since the final comparison and the arguments are not sensitive to them and also because of the uncertainties involved in the parameters of the problem. Taking $Z/d = 10$ and $Re \approx 10{,}000$ we get from the above mentioned experimental data $m = 0.5$, $n = 0.5$ and $C = 0.2372$. Then

$$Nu_0 = 0.75\ (Re)^{0.5}\ (Pr)^{0.4}$$

or

$$(h_0)_w/(h_0)_{st} = (k_w/k_{st})(\nu_{st}/\nu_w)^{1/2} (Pr_w/Pr_{st})^{0.4}.$$

Equipped with this expression, we follow the same procedure as in the case of flow over a flat plate – ignore condensation from steam and then take up two cases:

Case I. Comparison for same v_0

$$(h_0)_w/(h_0)_{st} = (28.2)\ (8.4)\ (1.215) \approx 288.7.$$

Case II. Comparison for same Re

$$(h_0)_w/(h_0)_{st} = (28.2)\ (1)\ (1.215) = 34.3$$

As mentioned before, steam jet velocity should be 70.9 times water jet velocity to maintain equal Re. In either case the factor 28.2 is due to high k_w, 8.4 due to low ν_w which is due to high ρ_w and 1.215 due to high Pr_w.

Steam jet impingement with condensation on wall

Let us try to estimate the effects of latent heat now and see by what factor it enhances heat transfer to the plate (skin). Condensation on the skin is a complicated process but we will make a simple assumption here that allows us to calculate this. Let

Q = Flow of heat energy in steam above skin (plate) temp. (in W)

$$= (\rho v_0 A_j)\ (\Delta T) C_p,$$

where $A_j = \pi d^2/4$ = jet exit area, m = mass flux $= \rho v_0 A_j$ and

q = Heat flux to the plate (in W/m^2)

$$= h\Delta T.$$

In the expression for Q, we have ignored the latent heat term since Q is a reference value from no condensation case. If we consider $A = \pi d^2/4$ as a reference area on the plate which is equal to the jet exit area (it is by no means critical),

$r = qA/Q$ = fraction of heat energy of jet transferred to this area.

Now the assumption is that the same fraction r of m is going to condense on this reference area A. Then the condensed mass flux $m_{\text{cond}} = rm$ (in kg/s), and the heat flux due to latent heat by steam condensation

$$q_{\text{cond}} = m_{\text{cond}} H/A = rmH/A.$$

Substituting the proper expressions and the numerical values

$$q_{\text{cond}}/q = H/(\Delta T C_p) = 16.47.$$

This ratio means that heat released due to condensation is about 16 times the heat transfer from steam if there were no condensation, other parameters being the same. This factor even though looks large is still smaller than the ratio $h_w/h_{st} = 34.3$ for even the case of same Re. For the same water and steam velocity h_w/h_{st} has a much higher ratio of 288.7.

More about condensation

We have purposely avoided till now considering details of the process of condensation itself. Even in the previous section an *ad hoc* assumption was made. Condensation depends on several factors and hence a direct comparison for the present purpose is not possible. Condensation on a surface takes place is the surface temperature is below the saturation temperature of the vapour. If the condensed liquid wets the surface, like water on skin, film condensation takes place, as against dropwise condensation. Dropwise condensation leads to a higher heat transfer rate. In either case the latent heat of condensation is imparted to the surface. The surface temperature goes up unless it is taken away by conduction or other means.

If steam at saturation temperature T_s condenses on a plate at uniform and steady temperature T_{wall}, a liquid film develops on the wall. The interface between the liquid film and steam is at temperature T_i which is only slightly lower than T_s. The rate at which condensation heat transfer takes place by film condensation on a vertical plate is given by the Nusselt formula

$$h_f = \left[\frac{g\rho_w k_w^3 H}{4\nu_w (T_i - T_{\text{wall}})Z}\right]^{1/4}.$$

Here Z is the distance measured down from the top edge of the plate.

For the interfacial heat transfer (which should be equal to the transfer in the film to the wall in the 1-dimensional model) we have the expression

$$h_i = \frac{q}{T_s - T_i} = \frac{2\sigma}{2-\sigma}\frac{H^2\rho_{st}}{\sqrt{2\pi R T_s^3}},$$

where R is the gas constant for steam and σ is the condensation coefficient. It may be mentioned that inert gases like air mixed in the vapour greatly inhibit condensation and hence heat transfer. Since $h_i >> h_f$, the total resistance to condensation heat transfer

$$\frac{1}{h_{\text{cond}}} = \frac{1}{h_f} + \frac{1}{h_i} \approx \frac{1}{h_f}.$$

Then,

$$h_{\text{cond}} \approx \left[\frac{g\rho_w k_w^3 H}{4\nu_w (T_s - T_{\text{wall}})Z}\right]^{1/4}.$$

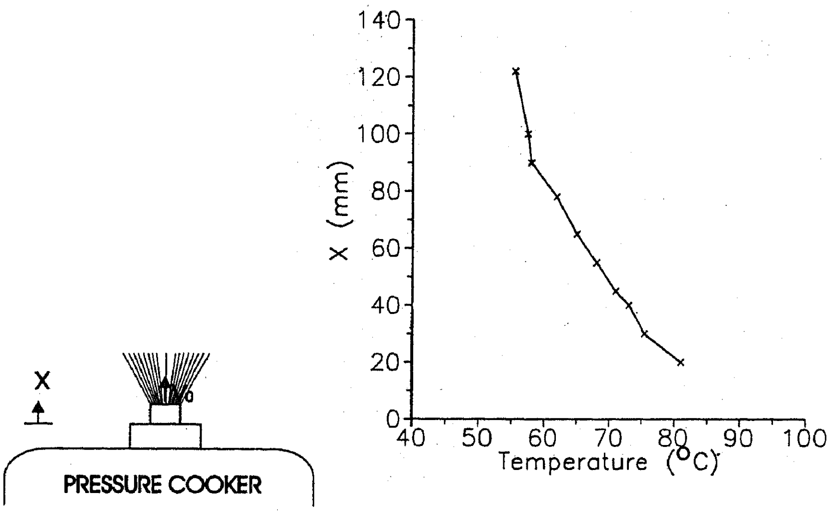

Figure 3. Temperature along the axis of a steam jet of 5 mm exit diameter.

This has a singularity at $Z = 0$ which is a modelling artefact. Getting an average value of h_{cond} by integrating up to a length L,

$$\bar{h}_{\text{cond}} = \frac{4}{3} h_{\text{cond}}(L).$$

For $Z = 0.01$ m, we get $\bar{h}_{\text{cond}} =$ 12,850 W/(m^2°C).

See that it is not easy to compare it with the boiling water case unless we have all the details in both the cases, since the actual value depends strongly on the details. For the flow over a flat plate case, for example, $\bar{h} = 2h(L)$. Substituting the values for water we get $\bar{h} = 2{,}003\sqrt{u_\infty/x}$. If we take $u_\infty = 1$ m/s and $x = 0.01$ m, we get $\bar{h} =$ 20,030 W/m^2°C. Different accident models and numerical values lead to heat transfer rates where either case may have a higher value.

The actual case when the skin is exposed to steam is an unsteady one. To start with, there is no film of condensed water and hence the skin is exposed to a very large heat transfer given by the coefficient h_i above. But it is only for a very short time and the thin film of water formed retards the heat flow. As it gets thicker its resistance increases and also since the skin is getting hotter the temperature difference causing the heat flow decreases. The formula given above for numerical estimation pertains to the steady state case. But see that when the steady state is reached, the temperature difference will be much smaller than 66°C.

What does it mean?

Since the question posed was vague, we have tried to rationalize it, as best as it can be done. Latent heat of steam is very high. (A feel for the very high latent heat of evaporation of water can be obtained by calculating the ratio $H/C_p = 535$°C. Thus only one gram of steam condensing in 535 g of water can raise its temperature by 1°C.) But there are other factors too. Water has high thermal conductivity and density (hence low kinematic viscosity). Because these aspects counteract, a quantitative analysis is required. This is done by modelling and we see that for the jet impingement problem the heat transfer from boiling water is much higher than that from steam at the same temperature, provided steam does not condense. The amount of steam that condenses has been obtained by making some assumptions. Then heat transfer from the condensing steam increases by a factor greater than 16. This factor, even though large, is still smaller than for the case of heat transfer from boiling water.

A word of caution is required here. We should not take these factors literally because of the assumptions involved. The two factors 16.47 and 34.3 are not really far apart. They indicate that it is possible to adjust parameters so that the first factor (16.47 here) can even be greater than the second one, i.e. condensing steam does cause a more severe burn. But it does not always happen.

The factors calculated in the two model problems (Laminar flow over a flat plate and turbulent jet impingement) turned out to be almost the same for similar conditions. This indicates that the present comparison is not sensitive to the mathematical model used. But it should be noted that it is true only for a comparison for similar conditions. The value of heat transfer, on the other hand, depends very strongly on the magnitudes of the parameters like fluid temperature, fluid mass involved, contact time, mass and rate of steam condensation, fluid velocity, whether the flow is laminar or turbulent, etc. The accident causing a burn is likely to be an unsteady process of a short duration, the values of the parameters being peculiar to each case. *That is why modelling this problem and drawing any general conclusions are not possible.*

On the other hand, there are some aspects on which we may comment. Steam jet usually has a higher velocity than that of a water jet. Water sticks to the body (unlike steam) thereby causing a longer contact. Steam that is issuing out in the form of a jet expands when it comes out and later the ambient air mixes with it which is called 'entrainment'. The entrainment cools the steam jet. This is shown in Figure 3 where I have plotted the temperature along the axis of a steam jet of 5 mm exit diameter. The steam jet was created from an ordinary pressure cooker by removing the weight. The bulb of the thermometer used was rather large and hence the measured values of temperature are likely to be lower than the actual values, the errors being larger closer to the exit (small X). Despite its crudeness, the experiment is adequate for the present demonstration.

Hot tea (temp ~60°C) can cause a severe burn (believe me). But how is it that we drink it and even enjoy its warmth? It is because we have mastered taking a sip that comes in the form of a sheet and gets cooled by air before coming in contact with the lips. Try drinking it with a straw; but be careful, you may burn your tongue.

It has not been verified experimentally or otherwise that steam causes a more severe burn than boiling water. But such notions persist because we get conditioned at a weaker moment, say when we study a chapter on latent heat. To remove such an ill-founded question, even though there is no supporting evidence, from mass circulation is not easy. It is because of the difficulties involved in getting a convincing quantitative answer when several factors are involved.

The problem we are discussing may sound innocent, specially because the *expected* answer sounds convincing, is simple to give and is uniformly accepted. A more serious problem is faced by undergraduate students while conducting experiments involving physical systems. Often there are many parameters that contaminate the experiment. The students do not have a good understanding of these extraneous factors, leave aside a good control over them in the experiment. These become a more serious handicap than the inaccuracies in the instruments. But the students are obliged to get an 'expected' answer and often 'cook up' the results. Experiments in the chemistry curriculum, in my experience, are usually better controlled and one becomes confident after successfully getting the answers.

Coming back to the question on burns, it is not always simple to decide which of the two burns is more severe. And regarding the problem we are discussing, I checked with a couple of doctors what they have in the medical literature. They do not bother whether the burn is caused by steam or water. They go by a different classification of the burns. Probably they are forced to be more honest since there is a serious problem in hand.

It may be interesting to mention here about the damage due to cold where the heat is extracted from the body. Air at 1°C makes us uncomfortable but we can survive it. But a human being in water at 1°C cannot survive it for more than 30 minutes. This is because of higher density, thermal conductivity and specific heat of water as compared to those of air. The question of latent heat does not arise here.

Does steam cause a more severe burn than boiling water? It may; it may not also. The question is not proper. I do not give an answer, but I have an explanation.

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Remote sensing application for delineating coastal vegetation – A case study

Satellite remote sensing has progressed rapidly and has provided a wealth of information on land as well as near-shore regions due to its repetitive and synoptic coverage. Remote sensing data have been used extensively worldwide for mapping coastal vegetation[1-3]. Similar work, however, along the Goa coast, is reported to a lesser extent[4,5].

This study envisages the use of digital image processing techniques for delineating geomorphic features and associated vegetation, including mangrove, along the central part of the Goa coast which includes the Mandovi–Zuari estuarine complex (Figure 1).

The typical geomorphological features, supported by favourable water currents and salinity, decide the lateral growth of coastal vegetation. Hence, identifying suitable geomorphic sites along the coast and the estuaries is a major task in depicting and delineating the vegetational features.

Multi-spectral scanner (band 4,5,6,7) data pertaining to scene (Path 49, Row 142) dated 31 March 1986 of Landsat 5 were used. Sub-image (Figure 2) with 900 lines and 900 pixels, covering Mandovi and Zuari estuarine complex, was separated and digital processing was carried out using DIPIX image processing system.

Because the influence of the coastal features on each band is highly correlated, compressing all bands into a single plane improves the identification of individual features. For this purpose,

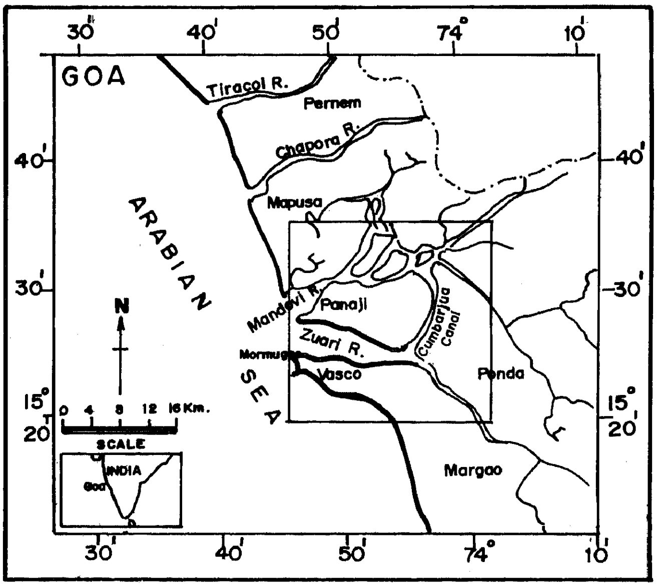

Figure 1. Location map of the study area.

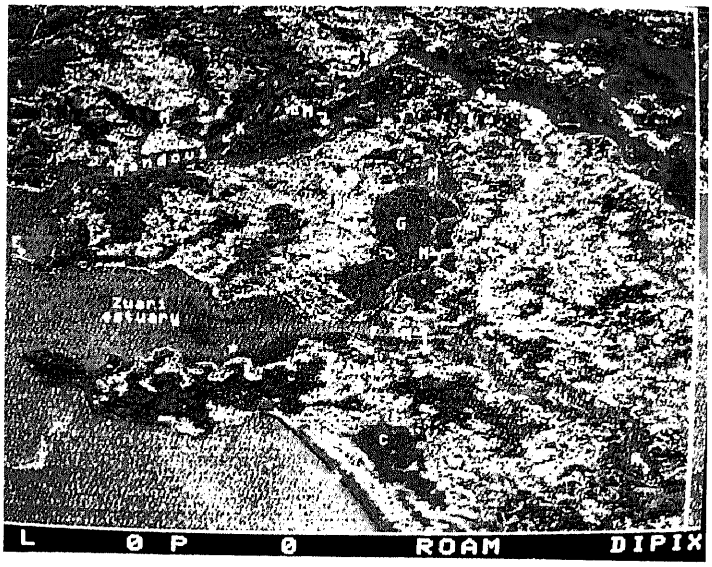

Figure 2. Colour composite image of first three principal components – PC1 (blue), PC2 (green), PC3 (red). The image shows some of typical sites of the following features. A, Green dense vegetation; B, Thin vegetation; C, Uncultivated field; D, Sandy beach; E, Rocky island; F, Sand bar; G, Mud flats; H, Water-logged body; I, Vegetated river terrace; J, River terrace devoid of vegetation; K, Point bar; L, Submerged land; M, Typical mangrove vegetation sites.

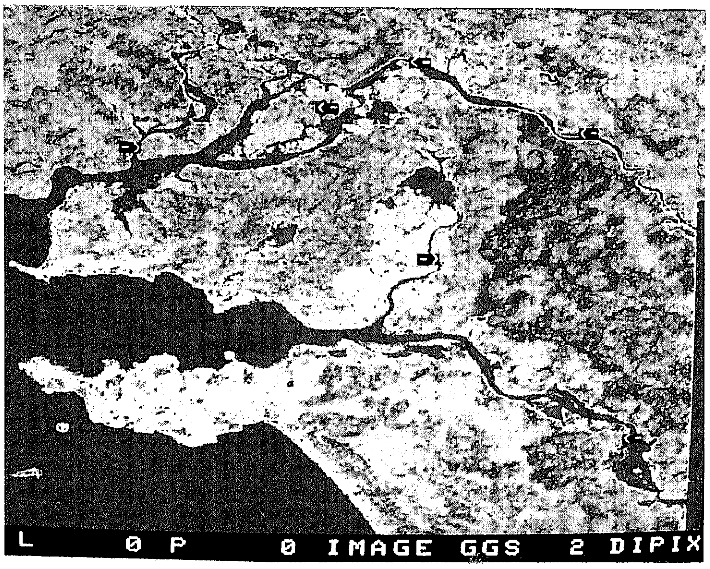

Figure 3. Intensity-modulated pseudo biomass index image showing typical mangrove sites (directed arrows).

Principal Component Transformation – digital processing of four spectral bands – was performed to produce four new Principal Component (PC) images[6]. Feature images of PC1, PC2, PC3 and PC4 were created on the monitor and photographed. False colour composite was generated from the first three principal components on the monitor and were photographed for visual interpretation.

Intensity-modulated pseudo biomass index image was generated by subtracting radiance values of 5th band from the radiance values of 7th band and dividing this by total radiance in both these bands. Three separate files were created by stretching and distributing gray values as described by Donker and Mulder[7], and stored using the same look-up table. The primary colours red, green and blue were assigned to first, second and third files respectively and finally Intensity Modulated Pseudo Colour Biomass Image was created and photographed.

All image enhancement products which include false colour composite, colour composite of PCs, intensity-modulated pseudo colour biomass and first two principal components images PC1 and PC2 were visually interpreted on the basis of tone, colour, texture and their association with other features. Shore line, estuaries and inter-tidal zones were extracted from the colour composite image. River terraces, mud flats, sand bars, point bars, meander bars, beaches, etc. were mapped from the PC colour composite image (Figure 2). These units showed reddish colour if barren and violet if they were wet. Vegetation on PC composite image is noticed by yellow green (spares) through green (dense) colour. Red colour in biomass index image marks the appearance of vegetation. The density variation was noticed on the basis of texture and colour shades, red (dense vegetation) to red spots on yellowish background (thin vegetation) (Figure 3).

Mangrove vegetation is extensive in the estuaries of the Mandovi and Zuari rivers, particularly in the silted-up fringes of the Cumbarjua Canal (Figure 3). Above the tidal limits, wherever the elevation allows beach formation, typical beach flora occur with shrubs and maritime grasses along with coconut plantations (site 'B' in Figure 2). This beach flora appears as a thin red line alongside the beaches, which appear as yellow strips on biomass image (Figure 3).

When the findings from the satellite data were compared with aerial photos (1960, 1:15,000 approx. and 1973, 1:25,000 approx.) and recent Indian Remote Sensing Satellite (IRS) images (path 96, row 62, L3 dated 5 April 1996) visually, it was noticed that small islands along the rivers are growing peripherally due to heavy siltation. Alluvial deposition along the banks of rivers have converted parts of bays into swamps. It is possible that the sediments are mainly derived from the inland mining rejects, and from heavy erosion due to deforestation in catchment areas of the rivers. These sediments are transported downstream as suspended matter. Under favourable hydrographic conditions, the sediments flocculate near the islands of low lying areas and give rise to submerged mud banks. As the sedimentation continues, these mud banks grow above the high tide level and are subsequently stabilized when floating vegetation hold the roots. Such mud banks thus form favourable ground for the luxurious growth of vegetation.

It is concluded from this study that i) repetitive coverage and spatial resolution of space borne data permits us to locate recently deposited alluvium and change in vegetation; ii) though constant biotic pressure is severely degrading the coastal vegetation, there are a few locations, where recent alluvium has deposited, forming favourable grounds for growth of vegetation.

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A rapid method of estimating essential oil content in heartwood of *Santalum album* Linn

Sandal (*Santalum album*) tree is mainly exploited for its scented heartwood which gives fragrant sandalwood oil upon steam distillation. Oil yield varies from 2.5 to 6.2% depending on age of the tree, soil, climatic and genetic factors. Fixative property and tenacious aroma of sandal oil are due to its major odoriferous sesquiterpenic constituents – alpha and beta santalols, forming about 90% of oil. However, alpha and beta santalenes and santalyl acetate which form about 6% of the oil also contribute to some extent to the overall characteristic odour of oil[1-4].

Sandalwood oil is generally obtained by hydro or steam distillation of powdered heartwood. A minimum quantity, 50 g of heartwood powder, is required for estimation of oil content. Estimation of oil in a small quantity of heartwood powder is difficult by the conventional steam distillation method. Two laboratory methods based on colour of wood and electrophoretic technique were developed earlier. In the first method, the colour of heartwood powder, whether light brown, yellow, brown or dark brown was determined by a tintometer and correlated with oil content[5]. Peroxidase isoenzyme activity in living bark tissue was determined by polyacrylamide gel electrophoresis and used as a marker in the second method[6]. Determination by tintometer method requires clear physical distinction of colours of heartwood powders and sometimes distinguishing colours of heartwood material in core samples taken from standing trees was found difficult.

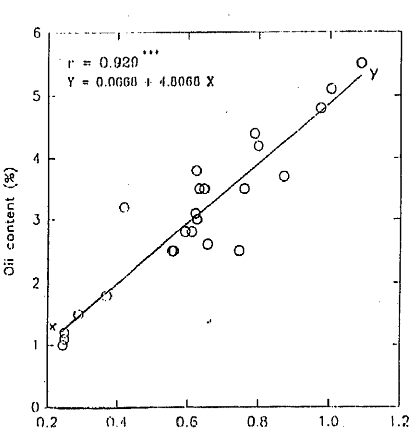

Figure 1. Relationship between oil content and optical density of hexane extract of sandalwood powder.

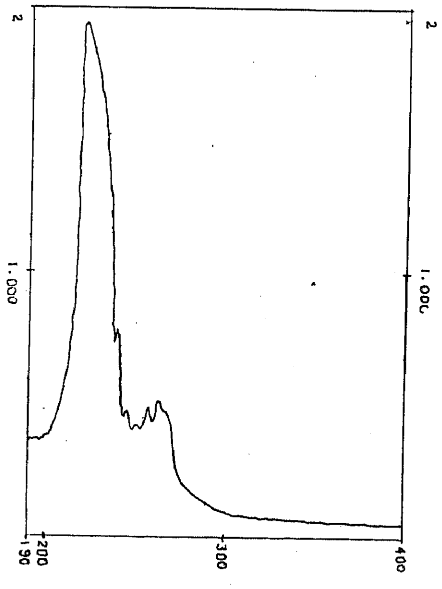

Figure 2. UV spectrum of sandalwood oil (25 mg/100 ml).

Table 1. Oil content (%) in 25 samples of sandalwood as determined by steam distillation and optical density (OD) measurement methods

	Oil content	
OD at 219 nm	Steam distillation method	By OD measurement
0.631	3.5	3.1
0.592	2.8	2.9
0.417	3.2	2.1
0.250	1.1	1.3
0.873	3.7	4.3
0.745	2.5	3.6
0.645	3.5	3.2
0.788	4.4	3.8
0.623	3.8	3.1
0.620	3.1	3.0
0.655	2.6	3.2
0.290	1.5	1.5
0.370	1.8	1.8
0.610	2.8	3.0
0.625	3.0	3.1
0.798	4.2	3.9
0.758	3.5	3.7
1.004	5.1	4.9
1.091	5.5	5.3
0.250	1.2	1.3
0.245	1.0	1.2
0.560	2.5	2.7
0.975	4.8	4.7
0.410	2.0	2.0
0.555	2.5	2.7

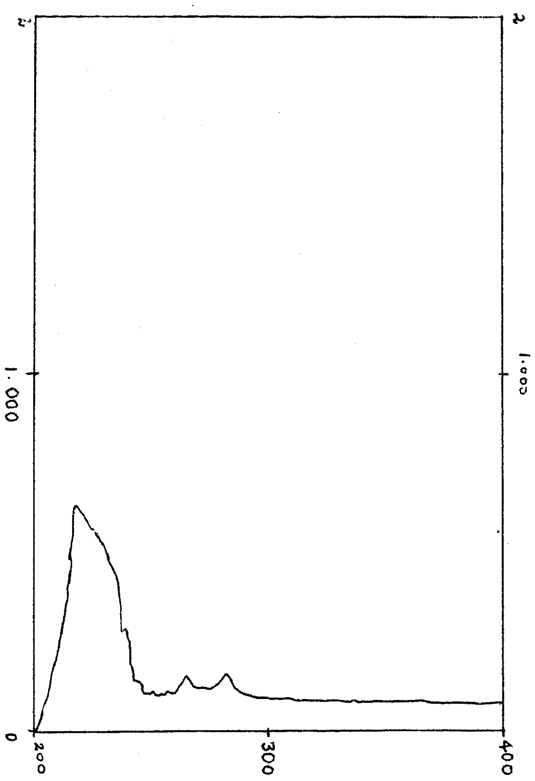

Figure 3. UV spectrum of hexane extract of sandalwood (100 mg sandal powder/100 ml).

Determination by polyacrylamide gel electrophoresis is based on enzyme activity of living bark tissue, which needs to be estimated within a few hours of sample collection which is not feasible when the collector of samples is on tour. The method highlighted in the current paper however, can be carried out later in the laboratory at any point of time after the sample collection. Also this method is simpler and quicker than the other two.

In the present study, estimation of sandal oil in a small quantity of heartwood material containing atleast 1 mg of oil is rendered simpler, quicker and fairly accurate by using UV spectrophotometer, measuring optical density (O.D) at 219 nm (max) on hexane extract of sandal powder, whose oil content was predetermined by steam distillation method.

Twenty-five samples of sandalwood were selected from sandal depots of Karnataka and Tamil Nadu at random. Heartwood was powdered after separation of sapwood and bark. 100 mg of heartwood powder was taken in 100 ml volumetric flask, hexane (60–70°C) was added up to the mark and kept aside for 18 h with periodic shaking. The supernatant was taken in quartz cell and OD at 219 nm (max) was measured by UV spectrophotometer (SHIMADZU-240). Oil content in the same sample of wood was also estimated by steam distillation of 100 g of powder separately. By keeping the heartwood powder in hexane for 18 h, it was found that the extraction was exhaustive and durations less than 18 h were found to be insufficient. The results are presented in Table 1.

Oil content was related with OD (Figure 1) by the regression equation

$$Y = 0.0668 + 4.8068\,X$$
$$(X = \text{Optical density})$$
$$Y = \text{Oil content (\%)}.$$

There was a highly significant and positive correlation between O.D and oil content ($r = 0.929$). UV spectra of sandalwood oil (25 mg/100 ml hexane) and hexane solution of one of the sandalwood powders at 219 nm (max) are presented in Figures 2 and 3. It is concluded that this method would be useful in rapid screening of sandal plants for their oil content and in selection of plus trees among different provenances.

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A more refined method to identify core institutes: A case study of biomass research

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Core institutes engaged in research on a given topic are those that contribute the most to published literature on that topic. As such, it appears easy enough to identify the core institutes: all one needs to do is to scan one or more abstracting services that cover the research field in question, note the institutional affiliations of authors, and prepare a tally – when the institutes are arranged in decreasing order of the number of papers contributed by them, we get a ranking order. However, in practice, the procedure turns out to be less straightforward.

IDENTIFICATION of major institutions engaged in biomass research the world over, and in India in particular, was one of the objectives of this study. The purpose of this exercise was to identify the institutions that have been actively contributing to the body of literature in biomass, in the period under investigation. Specifically, the top 50 institutions were to be listed and ranked. An exercise of this kind, it is hoped, will help in identifying institutions that are contributing extensively to the advancement of knowledge. The strategy used to achieve this objective is outlined below.

Conventionally, author-affiliation is used to identify major institutes/organizations. However, using author-affiliation as reported by the abstracting services has two disadvantages: (1) each abstract may not always provide complete affiliation (in the present study, the percentage of entries giving complete affiliation was not the same for each service *[Biomass Abstracts (BA), Chemical Abstracts (CA), Energy Abstracts (EA), Energy Abstracts for Policy Analysis (EAPA), Energy Research Abstracts (ERA), Forestry Abstracts (FA), Fuel and Energy Abstracts (FEA), Forest Products Abstracts (FPA), Indian Energy Abstracts (IEA), and Indian Science Abstracts (ISA)]*, it varied between 80 and 97%) and (2) eliminating the same research paper abstracted by two or more services was not possible (at least in the above approach).

The question, therefore, was whether one could use the author-affiliation reported by abstracting service(s) to identify core organizations in any field of interest for a given time span. An attempt has been made in this study to examine this question and explain the rationale behind its solution. Ideally, such a ranking should be done by obtaining exhaustive lists of published literature from, say, 100 prominent institutions. The selection of these institutions could be based on experience in the subject field. This list could then be narrowed down to the required 50 institutions. This is, however, impracticable and time-consuming. An alternative method is to scan all primary sources of literature published in this period. This, too, has its obvious drawbacks. The next choice is to use the information provided by abstracting services. However, using abstracting services to arrive at such a ranking is not as straightforward a task as it seems. The reasons for this are explained below.

Rationale

An abstracting service provides abstracts of only a portion of all the literature published in a given period. The need for, and the extent of, sampling is governed primarily by the policy of the service, and space considerations. For this reason, it is not advisable to use a single abstracting service to identify the core institutions. By considering many services, the coverage is increased but a complete coverage cannot still be guaranteed. However, using many abstracting services leads to a problem, the crux of which is this: the probability that the title of a primary (paper) document will be mentioned along with the complete affiliation of its author(s) is not necessarily the same for each service. This statement is based on the realization that the appearance of the name of the institution from where a paper is contributed is dependent on two factors, both of which are governed by chance. More importantly, the magnitude of these chances is not the same in all services. These two factors can be framed as questions: (1) What is the chance that a paper (bearing the affiliation

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This study is based on the thesis accepted by the University of Poona, Pune, for the award of Ph D degree, April 1996.

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Table 1. Sample selected for the study

Service	1982	1983	1984	1985	1986	Total
	Year of publication					
Samples of abstracts published						
BA	296	308	328	275	262	1469
FA	333	314	324	328	281	1580
FPA	168	143	152	144	111	718
						3767 (41%)
All the abstracts published						
CA	181	204	190	175	140	890
EA	118	91	163	137	72	581
EAPA	60	80	140	102	148	530
ERA	431	409	277	387	374	1878
FEA	159	110	86	94	99	548
IEA	60	71	139	150	48	468
ISA	65	40	25	290	124	544
						5439 (59%)

Table 2. Reporting pattern of complete affiliation in the field of biomass (all bibliographic forms)

Service	Total records	Complete address available	Percentage
BA	1469	1250	85.1
CA	890	773	86.9
EA	581	550	94.6
EAPA	530	465	87.7
ERA	1878	1703	90.7
FA	1580	1362	86.2
FEA	548	–	–
FPA	718	577	80.3
IEA	468	454	97.0
ISA	544	515	94.7

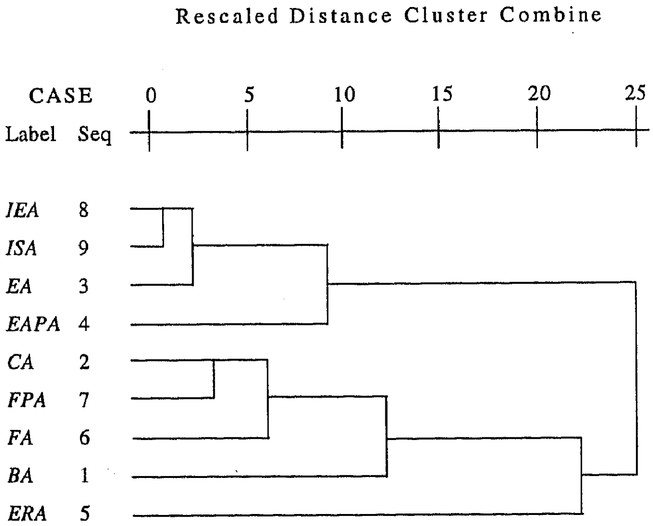

Figure 1. Dendogram using average linkage (between groups).

of the author) will be selected by a service? (2) What is the chance that, having been selected by the service, the complete affiliation will also be printed along with the abstract? If the selection of a paper is not dependent on either the service or the type of paper and if the probability of the complete affiliation of a selected paper being mentioned is the same for each service, this task would be simple: count the number of times each institution is mentioned (after eliminating duplicated records) in the sample of abstracts, disregarding the service that published the abstract. The reality is, however, different.

A simple example can illustrate what has been discussed so far. Suppose an article was published (with full affiliation) by ABC institute. The article was abstracted by two services S and T. Service S is known generally to mention affiliations in 90% of its abstracts, the corresponding figure for service T being 60%. Then it is 1.5 times more likely that one would have noticed the name ABC if one were to consult service S alone. But since abstracts of S and T are pooled, one would notice the name ABC if it is mentioned in S, or in T, or in both. The chance that this will happen is 96%. Now, consider another article published by DEF institute. It is abstracted by services M and N whose affiliation-reporting percentages are 50 and 80 respectively. By similar logic, it can be shown that the probability that DEF will be mentioned in M, or in N, or in both, is 90%. So if all the four services are considered, then the institute ABC is more likely to be mentioned than DEF, the difference in probability being 6%. The situation can get more complicated if one assumes that it is not equally likely that articles from ABC will be abstracted by services S and T. Similarly, one can assume that articles from DEF are not equally likely to be abstracted by services M and N.

There is nothing one can do to avoid the consequences of the fact that a service's coverage can only be selective and that it would be providing affiliations for only a certain fraction of the selected abstracts. But what one can do is to ensure that sets of services are chosen that consist of fairly similar services in these respects. For example, consider that 89%, 91%, and 92% of the abstracts in three services M, N, and P are those of the articles that have appeared in journals. The fraction of abstracts that carry complete affiliation is 75%, 77%, and 76% respectively in these three services. It can then be said that these three services are similar. Therefore, if two institutes – ABC and DEF – publish a journal article each, then it is equally likely that one would encounter their names. It is then immaterial which particular service published the abstract. That is, neither of the institutes will face a handicap. In other words, within a set of services there should be no comparative bias in selecting articles or reporting affiliation.

Methodology

A total of 9206 records were selected as a sample which forms 16.4% of the total output (56,240 abstracts) covering the period from 1982 to 1986 of the ten databases under examination. Stratified random sampling was employed in the case of *BA*, *FA*, and *FPA*. In the remaining seven services (*CA*, *EA*, *EAPA*, *ERA*. *IEA*, and *ISA*) original total (populations) of abstracts were taken for further analysis (Table 1).

Having selected the sample, the question that arises is: How to identify, from the ten services, those that are similar? In the present work, first the fraction of abstracts of journal articles where complete affiliation was provided by each service was ascertained. Overall, about 90% of the abstracts carried complete affiliation. There is no way of knowing how many abstracts in the remaining 10% did not record the affiliation because it was not furnished in the source document itself, and how many failed to do so during the abstracting process owing to human error, negligence, etc. It is assumed, for this exercise, that all primary source documents did carry the complete affiliation. The reporting of complete affiliation varied across the services, from 81 to 97%. A χ^2 test confirmed that reporting fraction was significantly dependent on service. By trial and error, it was possible to isolate two homogeneous sets (confirmed again by the χ^2 test) of services: one reporting affiliations to the extent of 94% and the other, 85%. One service, *EAPA*, still stood out (81%) when only journal articles were considered. It was then decided to consider all bibliographic forms and carry out a similar analysis (Table 2).

The overall reporting fraction in this case turned out to be 89%. The services showed a range from 80 to 97%. Again, it was possible to isolate two homogeneous sets. However, in this instance, *EPA* stood out (80%). It is only a coincidence that in both the exercises, the analysis yielded two sets. This then raised the question: Is use of only one criterion in forming sets of services valid? It was then decided to consider four other criteria: (1) the availability of affiliation in these services, (2) the number of journal titles monitored, (3) the number of conference titles monitored, (4) the number of languages, and (5) bibliographic forms (e.g. B for book, P for patent, J for journal article) considered by each service (Table 3).

It is hypothesized that these five factors primarily determine whether a given document will be selected for abstracting. A statistical technique, cluster analysis[1] was used to group similar services by simultaneously considering all the five criteria. The normal Z-scores of these five variables were used to prepare a 10×5 matrix for cluster analysis. The results showed that the ten services can be sorted into two homogeneous sets (Figure 1). The membership of each of these sets is also decided by cluster analysis. It would have been very fortunate if the results of cluster analysis had shown that all the services were similar and therefore part of a single set. But since

Table 3. Criteria considered for cluster analysis

Service	Availability of affiliation (%)	Journal titles monitored	Conference titles monitored	Languages considered	Bibliographic form(s) covered
BA	85.1	341	240	19	8
CA	86.9	213	69	19	8
EA	94.6	144	45	9	5
EAPA	87.7	78	65	4	8
ERA	90.7	38	218	12	11
FA	86.2	325	98	32	8
FEA	–	118	51	9	7
FPA	80.3	187	34	24	9
IEA	97	51	22	1	4
ISA	94.7	76	4	1	5

Table 4. Top 50 institutes in the field of biomass research

Institute (1)	Number of papers A(2)	C(3)	A′(4)	C′(5)	Rank A(6)	C(7)	A′(8)	C′(9)
Solar Energy Research Institute, Colorado, USA	120	129	144	157	1	2	2	3
Pacific North-West Laboratory, WA, USA	96	97	124	139	2	3	3	4
Institute of Gas Technology, Chicago, USA	71	85	79	107	3	4	4	5
Department of Agriculture, Washington, USA	62	62	221	236	4	6	1	1
Oak Ridge National Laboratory, USA	56	69	58	78	5	5	5	6
University of California, California	37	57	34	61	10	7	12	7
NAEMNDEN, Sweden	45	45	43	43	6	9	6	11
University of Florida, Florida	41	53	41	59	7	8	7	8
StatensEnergiverk, Sweden	40	40	38	44	8	10	8	10
Forest Research Institute, Dehra Dun, India	39	204	38	212	9	1	8	2
South Forest Experimental Station, USDA, USA	37	38	37	38	10	11	9	14
Argonne National Laboratory, USA	33	36	32	45	11	13	13	9
CSIRO, Australia	33	34	31	31	11	15	14	19
North Central Forest Experimental Station, USDA, USA	32	35	36	36	12	14	10	16
Tennessee Valley Authority, TN, USA	32	36	37	38	12	13	9	14
BatteleColumbus Laboratory, USA	32	36	35	45	12	13	11	9
Department of Energy, USA	32	36	31	38	12	13	14	14
Forest Products Laboratory, USA	30	32	27	32	13	16	16	18
Virginia Polytechnic Institute, VA, USA	30	32	27	35	13	16	16	17
Forest Research Institute, New Zealand	29	29	26	26	14	19	17	23
Texas A & M University, Austin, USA	28	37	31	39	15	12	14	13
Lawrence Berkeley Laboratory, Berkeley	27	31	30	32	16	17	15	18
University of Washington, Washington	27	27	25	25	16	21	18	24
CEC, Brussels	26	28	24	27	17	20	19	22
CEC, Luxembourg	25	26	26	28	18	22	17	21
Finish Forest Research Institute, Finland	25	25	22	23	18	23	21	26
Georgia Institute of Technology, Atlanta	23	25	24	24	19	23	19	25
INRA, France	23	29	24	28	19	19	19	21
Pacific Forest Research Centre, Canada	23	25	23	25	19	23	20	24
Swedish University of Agricultural Science, Umea	22	23	35	35	20	24	11	17
Forest & Forest Products Research Institute, Japan	17	17	19	15	25	30	22	32
Idaho University, Idaho	21	21	19	20	21	26	22	28
Auburn University, Auburn	19	19	16	17	23	28	25	31
Cornell University, Cornell	20	26	24	37	22	22	19	15
Indian Institute of Technology, Delhi	7	38	10	39	28	11	27	13
Central Institute of Agricultural Engineering, Bhopal	0	35	0	35	29	14	28	17
University of Arizona, Arizona	14	30	14	29	27	18	26	20
North Carolina University, North Carolina	18	26	16	23	24	22	25	26
Punjab Agricultural University, Ludhiana	0	26	0	25	29	22	28	24
University of Georgia, Georgia	16	20	18	18	26	27	23	30
Central Arid Zone Research Institute, Jodhpur, India	0	23	0	23	29	24	28	26
Illinois University, Illinois	16	23	17	21	26	24	24	27
Kerala Forest Research Institute, Peechi, India	0	22	0	19	29	25	28	29
National Research Council, Canada	19	22	18	24	23	25	23	25
University of Wisconsin, Wisconsin	18	22	16	23	24	25	25	26
Pennsylvania State University, Pennsylvania	19	20	16	25	23	27	25	24
Princeton University, Princeton	17	20	19	23	25	27	22	26
University of Hawaii, Hawaii	18	20	19	23	24	27	22	26
Forest Services, USA	30	38	32	40	13	11	13	12
Texas Tech University, Texas	18	18	18	20	24	29	23	28
Brookhaven National Laboratory, Upton, NY	18	19	18	20	24	28	23	28

that is not the case, the core institutions were identified separately with these two clusters/sets as well as with all of them pooled together.

The members of each group were also decided by cluster analysis. The three groups that were considered are: Group A: *BA, CA, ERA, FA, and FPA*; Group B: *EA, EAPA, IEA*, and *ISA*; Group C, which included all the services (excluding *FEA*). A card was prepared for each paper abstracted containing information on (i) name of the service in an abbreviated form (e.g. *BA*, *CA*) followed by year of publication and the abstract number, (ii) name of the first author followed by year of publication, (iii) title of document, (iv) source, (v) language of the text, (vi) affiliation of the first author (on

Table 5. Z-values calculated for Wilcoxon matched-pairs signed ranks test, for absolute values

	A	C	A′	C′
A	–	–5.5867 (0.0000)	–1.1293 (0.2588)	–5.4840 (0.0000)
C	–	–	–4.1795 (0.0000)	–2.8453 (0.0044)
A′	–	–	–	–5.5463 (0.0000)
C′	–	–	–	–

Figures in parenthesis indicate two-tailed probability, *p*.

Table 6. Z-values calculated for Wilcoxon matched-pairs signed ranks test, for ranked data

	A	C	A′	C′
A	–	–1.9663 (0.0493)	–0.3135 (0.7539)	–2.2524 (0.0243)
C	–	–	–1.6770 (0.0935)	–2.8117 (0.0049)
A′	–	–	–	–2.7504 (0.0060)
C′	–	–	–	–

Figures in parenthesis indicate two-tailed probability, *p*.

the back of the card), (vii) document type (e.g., B for book; J for journal article; P for patent), and (viii) country of the source document. For each group the cards were arranged first by country and then, within each country, by author. When names of authors were identical, titles of articles were checked. For the same research papers only one card (from any service) was retained and additional cards were kept aside. In the next stage, these cards were arranged by organization under each country. In the last stage, these cards were rearranged in decreasing order of productivity, irrespective of the country. Finally, a ranked list of core institutions (or ranked organizations) was compiled.

Results

The three ranked lists are fairly different. Group B places three Indian institutes first. SERI (Solar Energy Research Institute, Colorado; now known as National Renewable Energy Laboratory) appears in the 19th position. This cannot be the representative situation at the global level. In Group C, Forest Research Institute, Dehra Dun tops the list. Two Indian institutes are ranked at 12th and 19th positions. It is obvious that IEA and ISA have selected mostly Indian abstracts. This led to high ranking of Indian institutions. Group B thus could be used to identify the top 20 Indian institutes. Therefore, it was decided not to consider Group B for final comparative ranking. Table 4 shows comparative ranking for (1) Group A without duplicates, (2) Group C without duplicates, (3) Group A with duplicates (A′), (4) Group C with duplicates (C′).

The results show that comparative ranking does not change significantly if duplicate records are included. However, differences between Group A and Group C are significant. One can either compare the absolute values (columns 2, 3, 4 and 5) or the ranks (columns 6, 7, 8 and 9).

Comparison of absolute values using Wilcoxon matched-pairs signed-ranks test

From Table 5 it is evident that only the groups A and A′ are statistically similar. (Note that for four groups, there are six pairs that can be compared.) Groups are considered to be similar when *Z*-value is low and *p* is high.

Comparison of ranks using Wilcoxon matched-pairs signed-ranks test

By ranking the institutes it is observed from Table 6 that groups yield comparatively more similar results than for the corresponding absolute values (in each cell of Table 6, *Z*-value is lower and *p* value is higher than corresponding cell in Table 5).

It is also observed that the presence of duplicate records does not significantly alter the ranking, i.e. A′ = A and C′ = C. (Of course, duplicates create significant difference in absolute values between A′ & A.) From Table 6 it can be seen that the groups which are most similar are: A & C, A & A′, C & A′.

Conclusions

1. If absolute values of number of publications (the actual frequency distribution) are considered then it matters how one has grouped the services in order to identify major institutions.
2. If ranking is considered alone, then grouping of services is less critical. Also, duplicate records do not affect the ranks (but this is not true of absolute values).
3. The decision to select top 50 institutes out of the four groups, is to be based on experience and intuition, since Table 4 suggests that many of these groups produce similar ranking. Thus, we chose Group A (*BA*, *CA*, *ERA*, *FA*, and *FPA*). If top 20 Indian institutes are to be identified, then Group B (*EA*, *EAPA*, *IEA*, and *ISA*) can be used.

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Fossil woods and their significance

J. S. Guleria and N. Awasthi

Fossil woods are the most striking and frequently occurring megaplant remains in sedimentary rocks. They store a wealth of information about palaeoclimate, palaeoecology, phytogeography and evolution. Without them our knowledge about the arborescent plants of the past would have remained largely incomplete. They are of great economic importance as a source of energy since they form the major component of our coal and lignite.

XYLEM or wood is a complex tissue made up of more than one type of cells working together as a unit. It results from the activity of a special meristem, the vascular cambium, and constitutes the bulk of woody gymnospermous or angiospermous tree trunks. The xylem as a whole is meant to conduct water and mineral salts upwards from the root to the leaves and to give mechanical strength to the plant body. Xylem consists of (i) tracheids, (ii) vessels or tracheae, (iii) wood fibres, and (iv) wood parenchyma. The first two are called 'tracheary elements' and are concerned with conduction. They are non-living at maturity. Wood fibres mainly provide mechanical strength to the plant body and are usually dead cells. However, fibres with living protoplast and nuclei have been found to occur in many woody plants[1]. All the three xylem components are lignified and thick walled. Wood parenchyma is associated with storage of starch, oils and many other ergastic substances and consists of living cells. Sometimes parenchyma cells develop secondary walls and become lignified[1,2]. Wood is a fairly compact and hard material which resists decay and for this reason it often gets fossilized and is found most frequently in fossil records. The study of the anatomy of xylem or wood is called xylotomy.

Fossilization of wood

Wood may become fossilized in various ways though the main process is always by burial. The burial may be violent as happens in the case of volcanic eruption by lava flows or by extensive floods but usually it is gradual and gentle as may be observed in the peat bogs, lakes, rivers and along the sea shores. The burial material such as sand or mud slowly consolidates into rock. The wood under preservation often gets flattened forming coaly fossils called compressions. Due to flattening, the original structure of the wood in compression is usually crushed and is obscured. However, such flattening is generally prevented by the process of fossilization known as petrifaction and permineralization[3] in which the wood becomes partly or even completely replaced by minerals, usually by silica, in the form of opal, chalcedony or agate but sometimes by iron pyrites or calcium carbonate[3–6]. The wood is thus changed into stony fossils and unlike in compressions, the original shape and internal microscopic structures of such woods are well preserved. Petrified woods allow sectioning and examination of the tissue under microscope. Many petrified woods such as those of Deccan intertrappeans are preserved in this way. Internal structure may also be preserved perfectly when wood is fossilized as a charcoal-like material called 'fusain' which was probably formed by charring of trees due to forest fires. Petrifactions are the most frequently occurring type of fossil woods (Figure 1 *b*, *d*). Their occurrence in the arid and treeless landscape of Kutch and Rajasthan is of particular significance as a striking reminder of dense forests which must have flourished there millions of years ago. Sometimes we get moulds or casts of fossil woods. They are in a way the negatives of the plant material. The mould faithfully reproduces the surface features of the stem or wood, such as the design of bark, knots, wound and leaf bases. However, if other sediments get into the cavity of the mould and solidify, a cast or a possible replica of the original material is formed. Moulds and casts depict only the external form of fossil wood (Figure 1 *c*). Bark is very rarely found intact in fossil woods. It is the first casualty in transportation and early degradation of wood. Presence of bark in fossil woods provides an evidence of quick burial. It also indicates that the fossil has not drifted too far from its place of origin and hence is of considerable significance in determining the provenance of fossil woods. The tree trunks and branches have been recovered from the high tide water marks of sea in the Piram island, off the coast of Bhavnagar in Gujarat. It seems that as these woods drifted into the ancient sea, they were attacked by wood eating animals such as marine wood borer, *Teredo*, a bivalve mollusc, before fossilization. The borings made by penetration of these worms are often seen preserved in the fossilized woods (Figure 1 *b*). These woods were often fossilized as petrifactions with iron pyrites. A large number of

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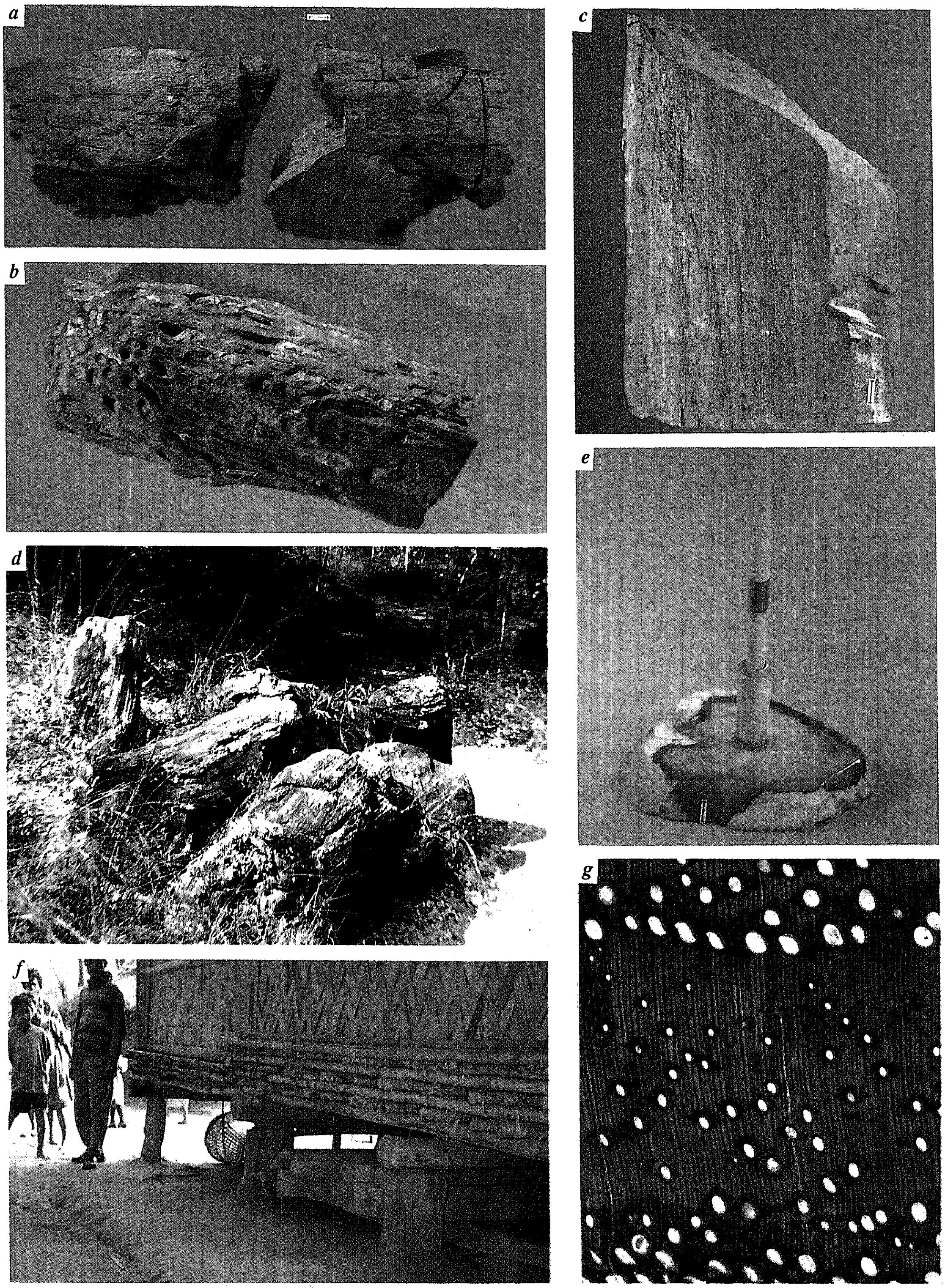

Figure 1*a–g*. ***a***, A carbonized wood sample from the Rajpardi lignite mine, District Bharuch, Gujarat; ***b***, A petrified wood eaten by marine wood borers (*Teredo*) from the Piram Island, off the coast of Bhavnagar, Gujarat; ***c***, A wood cast from the Paleogene sediments near Barmer, Rajasthan; ***d***, Petrified tree trunks lying scattered in a fossil wood locality at Tiruvakarrai in Tamil Nadu; ***e***, A decoration piece made of a fossil wood from Ginkgo petrified forest, State of Washington, USA (courtesy Dr Uttam Prakash); ***f***, Petrified wood logs used as support pillars/bases by villagers in their houses near Garobadha, Garo Hills, Meghalaya; ***g***, Transverse section of a fossil wood of *Lagerstroemia* (*Lagerstroemioxylon*) from Bikaner, Rajasthan, showing ring porous nature of the wood. (×15).

woods have also been preserved and described in the form of compressions from the lignite mines of India such as Rajpardi in Gujarat[7], Neyveli in Tamil Nadu and from the Tertiary sediments of Kerala and Maharashtra[8] (Figure 1 *a*) besides Andaman–Nicobar Islands[9]. Such type of woods have also been reported from the Plio-Pleistocene sediments of the Karewa lakes of Kashmir[10]. Sometimes calcified woods have also been found at different places. Pleistocene woods are also found sometimes in the inland lakes. Thus all kinds of fossil woods have been reported from India.

Identification of fossil woods

The identification of fossil woods requires detailed observation of their internal microscopic structures. Sometimes anatomical details are well preserved, particularly in cherty material. To study fossil woods, thin sections are cut in various planes such as transverse, tangential and radial, which help to build up the three-dimensional picture of the fossils as is the case for the study of extant woods. These sections are then ground, polished and examined under microscope. This method was first applied to petrified woods as early as in 1831 by Henry Witham and was followed by later palaeobotanists[11]. Anatomical features such as growth rings, tracheids, vessels, parenchyma, rays, fibres, resin canals and various kinds of minute pits, etc. are then examined (Figures 1 *g*, 2 *b–m*). Based on these characters, fossil woods can be identified and classified under various taxa and groups. Sometimes minute microscopic details are not preserved or remain obscure which makes it difficult to identify such woods to generic or specific levels.

Indian fossil woods

Oldest fossil woods from India come from the Palaeozoic sediments. A large number of fossil woods are known from the Lower Gondwana (Permian) formations of India. The vast forests of the Permian which covered much of Indian landmass during 245 m.y. –280 m.y. are represented in our coal seams by wood compressions and also by well-preserved petrifactions. All the known Indian Palaeozoic woods belong to Gymnosperms. The compression form is represented by *Vertebraria* Royle, referable to *Glossopteris*[12,13] irrespective of the fact of its being a stem or a root. The petrified woods have been described under various artificial genera, a comprehensive list of which has been given by Prasad[14]. These woods occur primarily in the Lower Barakar and Upper Raniganj and equivalent Kamthi formations of the Lower Gondwana. Surprisingly no wood has been reported from the Talchir, Karharbari and Kulti (Barren Measures) formations.

*Raniganj Formation/ *Kamthi Formation
Kulti Formation (Barren Measures)
*Barakar Formation
Karharbari Formation
Talchir Formation

(General sequence of Lower Gondwana formations of India, those containing fossil woods are marked by an asterisk).

Most of these woods have been reported from West Bengal (Raniganj Coalfield), Bihar (Jharia Coalfield) and Maharashtra (Kamthi Formation). A few records are also known from Andhra Pradesh, Madhya Pradesh and Uttar Pradesh (Singrauli Coalfield, Mirzapur District). Most of the fossils consist only of secondary wood but some of them possess homo- to heterocellular pith with sclerotic or secretory cells and endarch primary xylem. In these Lower Gondwana woods the secondary xylem is pycnoxylic, more or less uniform and mostly possesses araucarioid pittings but sometimes a mixture of araucarioid and abietoid pits along with taxinean spirals. The presence of well-defined growth rings in these woods indicates some climatic fluctuations. The xylem rays are homogeneous, generally 1–2 seriate, the cross field pits may be one to many, circular, bordered, araucarioid and taxodioid. These woods have often been found in coal-bearing beds in association with impressions of representatives of the *Glossopteris* flora. Although these woods are undoubtedly gymnospermous, their further affinities cannot be ascertained in the absence of any other supporting evidence. They could represent any of the orders like Glossopteridales, Cordaitales. Taxales and Ginkgoales. Gymnosperms were the most dominant group of plants in the Permian of India as has also been revealed by a large number of foliar taxa[15]. They continued their dominance throughout Mesozoic and Early Cretaceous. Birbal Sahni enhanced the significance of fossil woods by naming a new and enigmatic group of gymnosperms as Pentoxyleae after the characteristic wood genus *Pentoxylon* Srivastava (Figure 2 *a*, *b*) reported from the Rajmahal Hills of India[16], then considered to be Jurassic in age. The group Pentoxyleae has been accorded the status of an order Pentoxylales by later palaeobotanists. The age of the Rajmahal sediments has since been revised as Lower Cretaceous. The woods of Pentoxylales are remarkable in having poly 'steles' or many vascular 'bundles'[17]. *Pentoxylon sahnii* frequently had five steles. The name of the genus and the group was derived from this feature. Five was the commonest number of steles though the number varied. The secondary wood was compact and markedly eccentric with a much greater development towards the centre of stem with distinct growth rings (Figure 2 *b*). Although polystelic features of the stems show resemblance with Palaeozoic medullosan

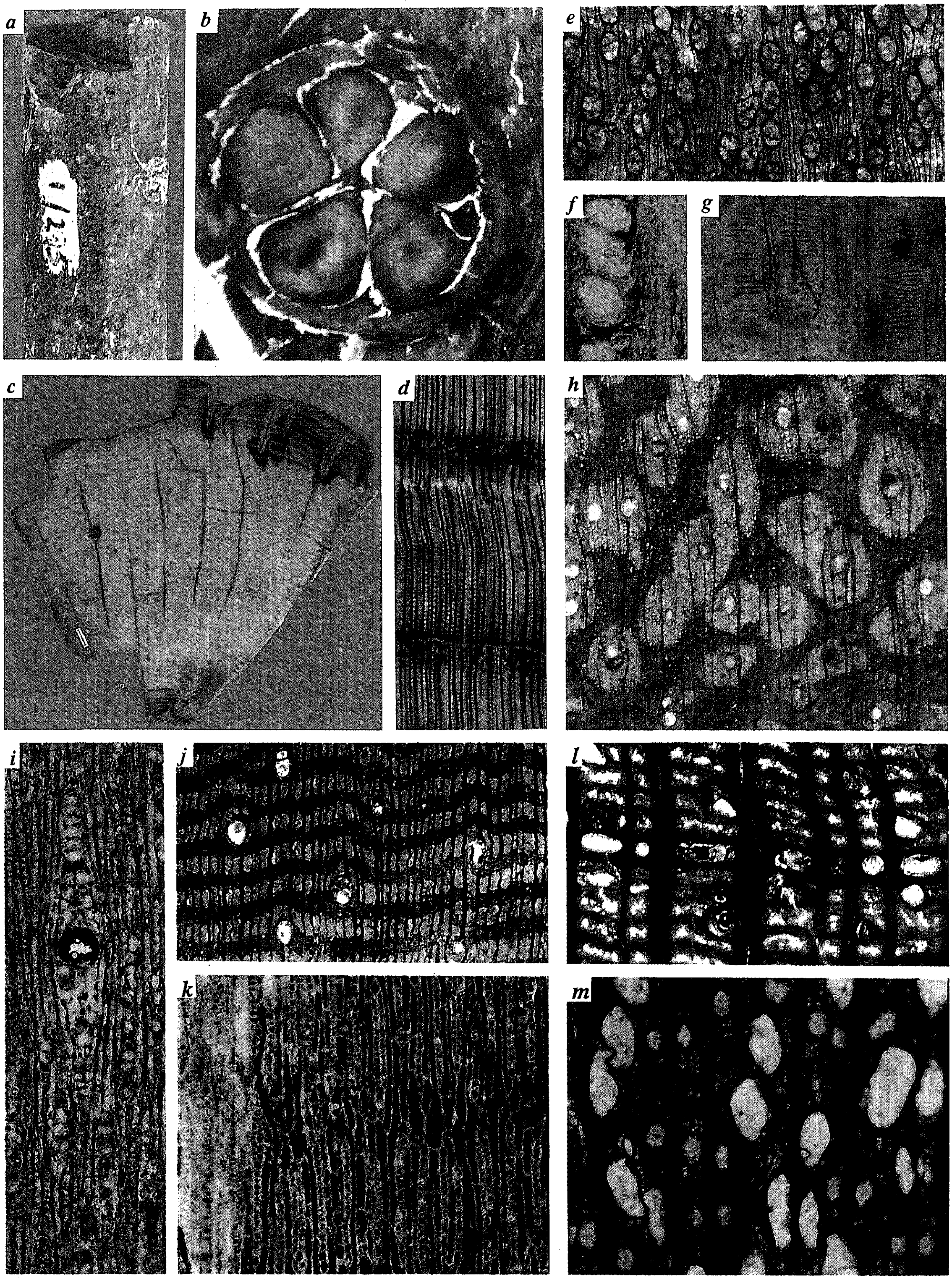

Figure 2 ***a–m***. ***a***, A specimen of *Pentoxylon sahnii* (× 2); ***b***, Transverse section of *Pentoxylon sahnii* showing polysteles, eccentric pycnoxylic wood and growth rings (× 10); ***c***, Transverse section of *Sahnioxylon rajmahalense* showing distinct growth rings (× < 1/2); ***d***, Transverse section of *Sahnioxylon rajmahalense* showing size difference in growth rings of early and late wood (× 15); ***e***, Transverse section of a fossil wood showing highly tylosed solitary vessels (× 20); ***f***, Tyloses in a vessel as seen in longitudinal section of a fossil wood (× 70); ***g***, Radial longitudinal section of *Hydnocarpoxylon indicum* Bande and Khatri showing scalariform perforations in the vessels (×100); ***h***, Transverse section of a fossil wood of *Isoberlinia* (an African genus) from Kachchh showing abundant paratracheal parenchyma (× 20); ***i***, Longitudinal section of a fossil wood of *Mangifera (Mangiferoxylon scleroticum* Awasthi) showing radial gum canal in the rays (× 70); ***j***, Transverse section of a fossil wood of *Millettioxylon indicum* Awasthi showing banded type of parenchyma (× 20); ***k***, Longitudinal section of *Millettioxylon indicum* Awasthi showing storied arrangement of xylem rays (× 70); ***l***, Transverse section of *Sterculinium kalagarhense* Guleria showing banded parenchyma and traumatic gum canals forming a row (× 20); ***m***, Transverse section of *Dipterocarpoxylon pondicherriense* Awasthi from Kachchh showing large solitary vessels and small axial gum canals (× 15).

ferns, they differ in having pycnoxylic wood as seen in gymnosperms.

The earliest records of flowering trees in India, in the form of a large number of petrified woods, have been reported from the Deccan Intertrappean sediments of Late Cretaceous–Early Tertiary of Central India of about 65 m.y. age. They represent varied dicot genera, even woody lianas, *e.g.*, *Aristolochioxylon*, and a number of palms[18] which are the characteristic components of any moist tropical forest. Similarly a large number of modern genera are represented by their fossil woods in the Neogene sediments[8,19]. Widespread occurrence of fossil woods of Dipterocarpaceae, the dominance of leguminous woods along with wood remains of Sapotaceae, Ebenaceae and Rosaceae help to demarcate the Neogene flora of India from the Palaeogene. Gymnosperms were rare during the Tertiary. With the advent of angiosperms they obviously declined fast and became a moribund group. They are represented by the rare occurrence of woods of *Araucarioxylon* and *Podocarpoxylon*, the two typical southern gymnosperm genera until the invasion of northern conifers in India such as *Abies*, *Cupressus* and *Pinus*, whose wood remains have been found in the Pliocene–Pleistocene deposits of Kashmir[10]. Thus the fossil woods are an important source of information in tracing the antiquity of extant arborescent plants.

Discussion

As stated earlier, wood provides mechanical strength to the plant body and conducts water and mineral salts from roots to leaves. These functions are vital to terrestrial life. Thus the development of woody or vascular plants was a critical factor in the initial conquest of land by plants around 410 m.y. ago (Ludlovian) during the Upper Silurian period. The evidence for this was provided by Obrhel[20] by his discovery of *Cooksonia* (*C. hemispherica*), an extremely simple plant consisting of small, naked or leafless, dichotomizing axes with slender cetrarch protosteles bearing terminal sporangia, from the Middle Ludlovian deposits of Bohemia, belonging to sub-division Rhyniophytina[21,22]. *Cooksonia* is thus considered to represent geologically the oldest vascular plant known to date. The vascular system consists of slender strand of annular tracheids[23]. The middle Devonian (Givetian, about 370 m.y. ago) period heralded the initiation of the cambial activity leading to the secondary growth (*Schizopodium*). Thus the anatomical studies of fossil woods has enabled us to understand how the pioneering land plants evolved from the earliest seaweed-like forms to some of the first large trees such as *Archaeopteris* and *Callixylon*, the pro-Gymnosperms which first appeared in the Middle Devonian about 380 m.y. ago[24]. Likewise polysteleic nature of *Pentoxylon* and related stems along with pycnoxylic wood have provided some kind of connecting link between the ferns (Medullosan type) and the gymnosperms.

The study of fossil woods has provided substantial data about palaeoclimate, palaeoecology, palaeogeography, phytogeography and evolutionary characters[8,18,19,25–34].

Analysis of growth rings in fossil woods provides information about palaeoclimate[35,36]. Depending on changes in radial cell diameter within the growth ring and the variation in the ring width, it is possible to extrapolate climate information. This study of tree rings to reconstruct past and present climate is called 'dendroclimatology' which is a subfield of 'dendrochronology'[37]. This kind of study has lately been seriously undertaken in our country[38,40]. Dendrochronological research could contribute to a better understanding of the dynamics of monsoon[41]. Growth rings are normally distinct and well defined in trees of temperate regions with pronounced seasonal changes. Accordingly, Permian to Early Cretaceous Indian woods show well developed and distinct growth rings (Figure 2 *b–d*) on account of palaeolatitudinal position of Indian Plate in cooler region at that time[42,43]. Growth rings are usually not well marked and indistinct in the woods of tropical trees (Figure 2 *e*, *j*, *l*) with a few exceptions like *Tectona grandis*, etc.[44–46].

The Late Cretaceous–Early Tertiary Deccan Intertrappean flora is mainly represented by fossil woods whose study has provided the bulk of information about the composition of this flora. A large number of its woody dicot genera such as *Artocarpus*, *Barringtonia*, *Canarium*, *Dracontomelum*, *Drypetes*, *Garcinia*, *Homalium*, *Lophopetalum*, *Polyalthia*, *Sonneratia* and *Syzygium* indicate that at that time tropical humid conditions prevailed over the Indian peninsula[47]. Somewhat similar climatic conditions extended into the later half of the Tertiary as is revealed by a large number of woods of the dipterocarpaceous taxa (e.g., *Anisoptera*, *Dipterocarpus*, *Dryobalanops*, *Hopea*, *Shorea*, etc.) along with those of *Calophyllum*, *Gluta*, *Cynometra* and *Afzelia-Intsia*. In addition to these, the occurrence of *Bauhinia*, *Cassia*, *Cordia*, *Mangifera*, *Ormosia*, *Sterculia*, *Terminalia*, etc., ranging from semi-evergreen to deciduous woody taxa in the Late Tertiary, indicates a gradual decrease in rainfall and evolution of drier conditions. Palaeogeographically the occurrence of fossil woods of *Sonneratia* in the Tertiary of central and western India and *Heritiera* in eastern India indicates the existence of coastal conditions far inland in the past than at present. Likewise fossil woods of *Lagerstroemia*, *Dracontomelum*, *Drypetes*, *Syzygium*, *Terminalia arjuna* and *Barringtonia* indicate the presence of water channels and damp or swampy conditions.

The occurrence of certain South American, African and Australian woods such as *Simarouba*=*Quassia*[48,49], *Hyphaene*[50] and *Eucalyptus*[51] in the Deccan Intertrappean (Late Cretaceous) sediments of Central India indicates the proximity of South America, Africa and Australia to India at that time. The occurrence of these taxa in India probably represents angiospermic remnants of the Gondwanaland flora. Similarly the presence of certain typical Malayan elements, largely represented by the woods of dipterocarpaceous genera[25] (Figure 2 *g*) and African taxa like *Isoberlinia* (Figure 2 *h*), *Entandrophragma*, etc.[52,53] in the Upper Tertiary sediments of India and their complete absence in the Deccan Intertrappean sediments indicate the invasion of such genera from the east and west respectively, after the establishment of land connections with India by early Miocene through Indonesia and Myanmar on one hand and via Ethiopia and Arabia on the other. Likewise the complete absence of woods or any other megafossils of northern conifers such as Pinaceae in the Miocene sediments of the Indian subcontinent indicates that these conifers entered into India after the uplift of the Himalayas at the end of Miocene when the conditions became cooler and conducive to their growth. The occurrence of a fossil wood of *Prunus* in the Miocene sediments of Ladakh[54] at a height of 4581 m indicates the uplift of the Himalayas by about 2445 m since then as *Prunus* does not grow above 2140 m at present. Based on detailed analysis of Indian fossil woods ranging from Deccan Intertrappeans to Neogene in age, Bande and Prakash[27] have been able to trace the evolutionary trends in the secondary xylem of dicot woods. Features like wide apotracheal parenchyma bands and storied structures of various elements such as rays, wood fibres and parenchyma are more advanced and hence they are found frequently in the younger Neogene woods in contrast to older Deccan Intertrappean woods (Figure 2 *j–l*). Similarly vessel members with scalariform perforations are more common in Deccan Intertrappean woods than in the younger Neogene woods since it is considered to be a primitive feature (Figure 2 *g*). Normal axial gum canals are present in the Neogene woods (Figure 2 *m*) whereas they are absent in the Pre-Neogene Indian woods.

Fossil woods are of great economic importance when they occur in large quantities as in the case of lignite and coal mines. Neyveli lignite is composed of large number of fossil woods and a number of carbonized woods have also been collected from the Rajpardi lignite mine of Gujarat[7,8] (Figure 1 *a*). Similarly Gondwana coals also consist of woody material[55] besides other vegetal remains. Such large deposits of lignites and coals are our major source of energy. In addition, petrified wood logs have been used by villagers as pillars and support bases for making their houses (Figure 1 *f*) in the north-east India and for marking the boundaries of fields in almost all other areas near fossil localities. They have occasionally been used as curios and for decorative purposes (Figure 1 *e*) after cutting and polishing.

The so-called fossil wood forests or parks with a large number of tree trunks as at Tiruvakarrai in Tamil Nadu (Figure 1 *d*), Mandla in Madhya Pradesh and Akal fossil park near Jaisalmer in Rajasthan, act as repositories of arborescent fossil plants and remind us of our luxuriant forests during the past.

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Adult crowding effects on longevity in *Drosophila melanogaster*: Increase in age-independent mortality

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The manipulation of longevity through environmental treatments has provided many insights into the physiological processes affecting life-span. Here, we report the reduction of longevity in adult *Drosophila melanogaster* after three days of moderate adult crowding. Crowding is shown to reduce life-span through increased age-independent mortality rather than through altering the rate of ageing. Preliminary evidence also suggests that increased age-independent mortality after crowding may result from a reduction in stored energy reserves. The results also suggest that populations of *D. melanogaster* routinely maintained at high-adult densities may be relatively less susceptible to the detrimental effects of crowding on longevity.

THE study of ageing has long been approached at two different levels of biological organization: at the individual and sub-individual level by gerontologists, and at the populational level by demographers. In the past one-and-a-half decades, evolutionary biologists have tried to unite these divergent approaches by trying to understand how and why ageing evolves, an approach that combines demography, population genetics and physiology[1-3]. From this evolutionary viewpoint on ageing, longevity (or life-span) is seen as but one among a multitude of life-history traits, all interrelated as a consequence of shared underlying physiological constraints[2-10].

Genetic and environmental manipulation of longevity

Many studies on ageing in the past several years have focused upon genetic and environmental manipulations of life-span[2,3]. For example, increased life-span has successfully been selected for in *Drosophila melanogaster* by several workers independently[1,11-13]. Similarly, environmental manipulations such as dietary restriction[6], restricted mating[14-16], and the addition of urea to the food medium[17] have all been observed to enhance longevity in *D. melanogaster*. Similar effects of many environmental treatments on longevity have also been observed in a variety of other invertebrate[7,18-21] and vertebrate[22-24] species. Moreover, investigation of the physiological mechanisms underlying observed genetic and environmental effects on longevity has provided useful insights into the nature of the constraints placed by correlations among life-history characters upon the attainment of increased life-span under a given set of conditions[2,5,25-27].

Estimating the rate of ageing

The demographic perspective on ageing suggests another level at which the nature of the effect of various factors on life-span can be assessed, by focusing on rates of ageing, rather than on changes in life-history traits correlated with longevity. In recent years, several studies have focused on 'rates of ageing' as reflected by the α parameter of the Gompertz equation[28] that models age-specific mortality as an exponentially increasing function of age[17,24,29-32]. According to the Gompertz equation, the mortality rate at age x, $\mu(x)$, is given by

$$\mu(x) = Ae^{\alpha x},$$

where the two parameters, A and α, represent the age-independent mortality rate and the rate of increase in mortality rate with age, respectively: this latter parameter, α, is considered to represent the rate of ageing[2,18,29-32]. Results of some studies on *Drosophila*[17,32] strongly suggest that environmental factors affecting longevity tend to do so through their effect on the age-independent Gompertz parameter (A), whereas genetically induced changes in longevity tend to involve changes in the Gompertz 'rate of ageing' (α). There is also some evidence that, in the bruchid beetle *Callosobruchus maculatus*, mating *per se* may affect the Gompertz 'rate of ageing', whereas egg-production affects the age-independent Gompertz parameter[18].

Adult density and longevity

Adult density is an environmental factor that is known to adversely impact a variety of fitness correlates in diverse species[33–39]. In *Drosophila*, adult crowding over a period of many days has been shown to significantly reduce longevity[33,37]. In the present study, we used a set of 15 populations of *D. melanogaster*, that had been subjected to life-stage-specific density-dependent natural selection in the laboratory, to seek answers to the following questions: (i) Can brief episodes of moderate levels of adult crowding significantly reduce longevity? (ii) Is the effect, if any, of adult crowding on longevity mediated through changes in the age-independent or age-dependent parameters of the Gompertz equation, or both? (iii) Are populations regularly maintained at high adult densities better able to withstand the deleterious effects, if any, of adult crowding on longevity?

Methodology

Experimental populations

This study used three sets of five replicate populations of *D. melanogaster* that had each been subjected to differing levels of larval or adult density for over 50 generations[40]. All populations were maintained on banana-molasses food at 25°C, continuous light, and had a generation time of about 3 weeks. Population sizes of each generation were of the order of 2000–4000 breeding adults. The five populations crowded as larvae ($CU_1 \ldots CU_5$) were reared at densities of 1000 or more larvae per 6 dram vial (2.2 cm d × 8.4 cm h). Eclosing adults were collected daily from these vials, and kept at a low density of about 60–80 adults per 8 dram vial (2.4 cm d × 9.5 cm h). The five uncrowded populations ($UU_1 \ldots UU_5$) were reared at low larval densities of 60–80 larvae per 8 dram vial; eclosing adults were subjected to the same density as the CU populations. The five populations crowded as adults ($UC_1 \ldots UC_5$) were reared at low larval densities of 60–80 larvae per 8 dram vial; eclosed adults were collected from these vials on the 13th day after egg-laying and kept in 8 dram vials at densities of about 160–200 adults per vial. The three sets of populations, thus, differed in the degree of larval or adult crowding to which they were exposed, with the UU populations acting as controls to both the UC and CU populations. Prior to initiating a new generation, all the eclosed adults from a population were dumped into a plexiglass cage (25.5 × 20 × 14.4 cm^3) and supplied with liberal amounts of live yeast paste for 2 days before egg collection. All three sets of populations were derived from the five B populations of Rose[1], each B population being used as the progenitor of one CU, one UC, and one UU population. Consequently, CU, UC and UU populations bearing the same numerical subscript are more closely related to each other, as compared to other populations subjected to the same density regime.

Collection of adult flies for longevity assay

Prior to initiating the longevity assay described below, all test populations were passed through one complete generation of identical rearing conditions, to eliminate any differences among selected lines due to environ-

Table 1. ANOVA for longevity of UU, UC and CU flies after three days of crowded (150 flies/vial) or uncrowded (8 flies/vial) conditioning (Cond). Longevity was assayed on flies kept in groups of 4 males and 4 females per vial. Significant fixed main effects and interactions are indicated in bold type (Blk, block, Sel, selection, Cond, conditioning treatment; parentheses are used to denote nested effects)

Source	*df*	MS	*F*	*P*
Block	4	1671.44	13.54	<0.0005
Selection	2	1246.85	1.59	>0.25
Conditioning	**1**	**4971.88**	**8.09**	**<0.05**
Sex	**1**	**39991.16**	**213.83**	**<0.0005**
Vial (Blk × Sel × Cond)	270	123.39	1.06	>0.20
Blk × Sel	8	782.73	6.36	<0.0005
Blk × Cond	4	614.69	4.99	<0.001
Blk × Sex	4	187.02	1.06	>0.25
Sel × Cond	2	350.29	1.69	>0.10
Sel × Sex	2	1038.40	3.35	>0.05
Cond × Sex	1	716.42	1.97	>0.10
Sex × Vial (Blk × Sel × Cond)	258	176.84	1.53	<0.0005
Blk × Sel × Cond	8	207.83	1.68	>0.05
Blk × Sel × Sex	8	310.70	1.76	<0.05
Blk × Cond × Sex	4	363.87	2.08	>0.05
Sel × Cond × Sex	2	152.79	0.50	>0.25
Blk × Sel × Cond × Sex	8	302.60	1.72	>0.05
Error	1525	115.92		

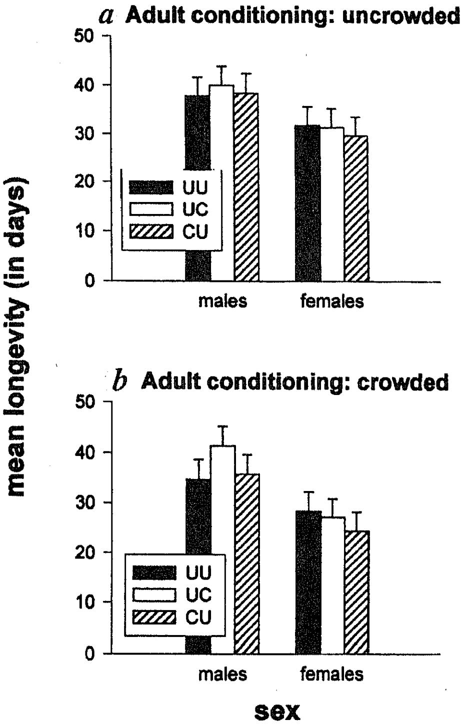

Figure 1. Mean longevity of flies after three days of uncrowded (*a*) or crowded (*b*) conditioning as adults. The error bars depict 95% confidence intervals about the mean of the five replicate populations of each selection regime, and were calculated using least squares estimates of the standard errors of cell means in the randomized block ANOVA.

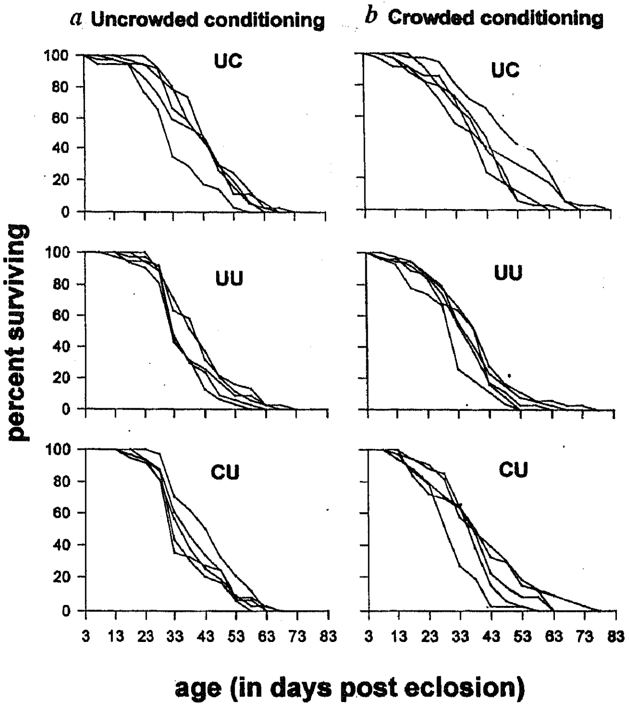

Figure 2. Per cent survival to various ages of adult males from each of the 5 replicate UU, UC and CU populations, after three days of high (150 flies/vial) or low (8 flies/vial) density conditioning.

mental or maternal effects. Eggs were collected from the adults of each stock population and placed in 8 dram vials at low densities of 60–80 eggs per vial. Eclosing adults from these vials were then collected into cages; eggs laid by these adults were collected and transferred into 8 dram vials at low densities of 60–80 eggs per vial. Adult flies eclosing in these vials were collected one day after eclosion, and subjected to one of the two conditioning treatments: crowded (75 males and 75 females per vial) or uncrowded (4 males and 4 females per vial). All conditioning vials contained exactly 5 ml of food medium. For each population, 7 vials were set up at high density and 15 at low density, adding up to a total of 330 conditioning vials (3 selection regimes × 5 replicate populations × (7 vials at high density, 15 vials at low density). The flies remained in these vials for 3 days, after which they were used for setting up the longevity assay described below.

Longevity assay

After 3-day conditioning period, flies were placed into vials with about 3 ml of food medium at a density of 4 males and 4 females per vial; 10 such vials were set up for each population × conditioning combination. Thus, a total of 2400 flies were assayed for longevity. The flies were transferred to fresh food vials every third day until all flies had died. All vials were checked daily for deaths. Dead flies in a vial were not replaced over the course of the assay. Life-span was measured as the time, in days, from the start of the conditioning to the day of death. The longevities reported here are, consequently, the conditional longevities of flies that survived the 3 days of conditioning.

Statistical analysis

The longevity data were subjected to an analysis of variance (ANOVA) using the procedure GLM of SAS for Windows version 6.08. Due to the pattern of relatedness among the CU, UC, and UU populations (CU_i ,UC_i ,and UU_i are more closely related to each other than either of them is to other populations with which they share the same selection regime, $i = 1..5$), sets of CU, UC and UU populations, matched by subscripted indices, were treated as random blocks in the analyses. Selection-regime and conditioning density were treated as fixed effects crossed within each block (our primary interest was in these fixed effects; block in this experiment confounds ancestry and handling effects and, thus, although we are interested in accounting for this variation by treating block as a factor, we do not try to interpret any significant random effects). Because the measurement of longevity was done on individual flies in each vial, the ANOVA model included vial as a random effect nested within the block × selection × conditioning interaction. Sex was treated as a fixed factor crossed with all the rest.

From the data on longevity, we also estimated the age-independent (A) and age-dependent (α) parameters of the Gompertz equation that models the age-dependence of mortality rates[28], using a maximum likelihood method utilizing untransformed survival data[31]. The estimates of A and α from each population ´ conditioning combination were then used as data for analyses of variance, treating selection-regime, conditioning and sex as fixed factors crossed within the 5 replicate blocks. In similar previous experiments we have tested the goodness of fit of the Gompertz equation to data on mortality collected from males and females of over 20 populations of *D. melanogaster* related to the populations used in this study under various environmental conditions and the fit has always been excellent with R^2 values consistently being above 90% (refs 17,31).

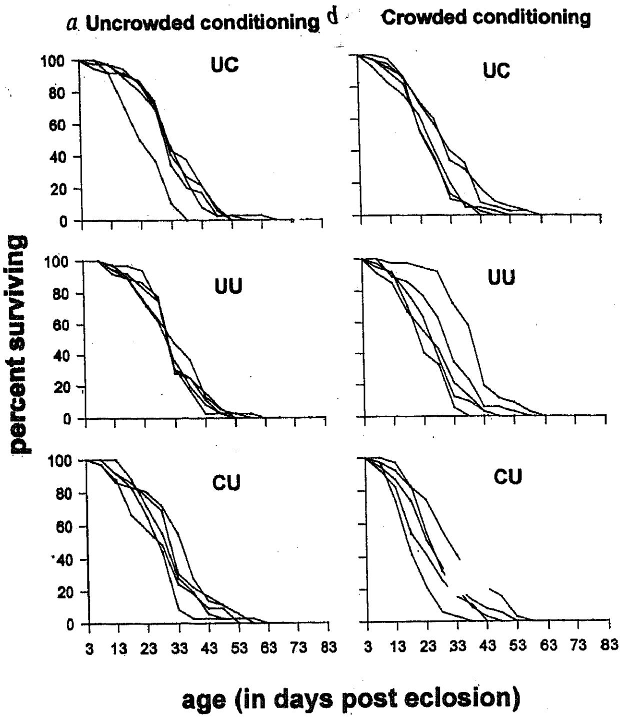

Figure 3. Per cent survival to various ages of adult females from each of the 5 replicate UU, UC and CU populations, after three days of high (150 flies/vial) or low (8 flies/vial) density conditioning.

Effect of adult crowding on longevity

The ANOVA results for longevity after 3 days of adult conditioning showed significant fixed effects of sex and conditioning density; selection regime did not have a significant effect (Table 1). In general, longevity decreased with increasing density of conditioning (an average reduction due to crowding of 2.75 days), and, as expected for *Drosophila*, males outlived females by an average of 9.2 days (Figure 1). Females were seemingly affected by crowding to a degree greater than the males: crowding reduced the longevity of females by an average of 4.2 days whereas in the case of males, the reduction was only 1.3 days.

Unfortunately, too much cannot be made of these differences because the early death of large numbers of females in the crowded treatment would tend to increase life-span in males due to reduced courtship and mating, thus counteracting to some degree the detrimental effect of the crowding treatment experienced earlier. Moreover, the degree and duration of crowding experienced by the populations in this study was moderate (mean mortality in our populations during the 3 days of crowded conditioning varied from less than 1% to 11.6%, whereas more severe crowding for 5 days in the same populations can yield mortality up to 90% (ref. 41). We were, therefore, more interested in broad patterns of mortality effects than in the specific magnitudes of the effects. Overall, there did not appear to be major differences in the pattern of effects of adult crowding on either mean longevity in the UU, UC and CU populations (Figure 1), or on the survivorship curves of males and females from these three types of populations (Figures 2, 3). The only significant difference among selection lines was that the mean longevity of UC males from the crowded conditioning treatment was greater than that of both CU ($P = 0.02$) and UU ($P = 0.01$) males. At a qualitative level, nevertheless, the per cent decline in mean longevity due to adult crowding was the greatest in the CU, and the least in the UC populations.

The ANOVA results for the age-independent (A) and age-dependent (α) mortality parameters of the Gompertz equation indicated that the crowded conditioning treatment lowered longevity through an increase in A. Of the fixed factors in the ANOVA model, sex, conditioning, and the selection regime × sex interaction were seen to have significant effects on the estimates of A, while only sex had a significant effect on estimates of α (Table 2). The reduced longevity of females, as compared to males, is well known in *Drosophila*[7,31], our re-

Table 2. Estimates of the age-independent (A) and age-dependent (α) mortality parameters of the Gompertz equation. The entries are the means (± 95 % confidence intervals) of the five replicate populations in each selection regime × conditioning combination. The only significant effects in the ANOVA were those of sex, conditioning, and selection regime × sex on estimates of A, and of sex on estimates of α.

	Uncrowded conditioning		Crowded conditioning	
Selection regime	Male	Female	Male	Female
Estimates of A				
UU	0.0026 (0.0010)	0.0030 (0.0012)	0.0031 (0.0015)	0.0043 (0.0019)
UC	0.0027 (0.0009)	0.0033 (0.0011)	0.0029 (0.0017)	0.0048 (0.0015)
CU	0.0025 (0.0010)	0.0035 (0.0010)	0.0035 (0.0008)	0.0071 (0.0025)
Estimates of α				
UU	0.0876 (0.0202)	0.1051 (0.0184)	0.0886 (0.0232)	0.1054 (0.0157)
UC	0.0807 (0.0124)	0.1022 (0.0218)	0.0729 (0.0190)	0.1015 (0.0325)
CU	0.0863 (0.0089)	0.0956 (0.0149)	0.0796 (0.0158)	0.0998 (0.0366)

sults show that this is due to both greater age-independent mortality (mean A in females was 0.0043, a 53% increase over the mean in males, 0.0028) and a more rapid rate of ageing (mean α in females was 0.1016, a 23% increase over the mean in males, 0.0826). The mean value of A in populations subjected to the crowded treatment was 0.0042, a 44% increase over the mean in the uncrowded treatment, 0.0029. The significant selection regime × sex interaction for estimates of A arose from the fact that means of selection regimes for the crowded and uncrowded treatments did not differ significantly in the case of males, but did so in the case of females (Table 2).

Conclusions

The results of the present study are consistent with the findings of previous studies that adult crowding reduces longevity in *Drosophila*[33,37]. Moreover, our results indicate that even brief periods of relatively moderate crowding can have a lasting effect on subsequent longevity (Table 1, Figure 1). Interestingly, it is also clear that, like other environmental factors affecting longevity in *Drosophila*[17,32], adult crowding affects longevity by altering the age-independent mortality rate rather than the rate of ageing *per se* (Table 2). The commonly observed difference in longevity between males and females in *Drosophila* is shown to be due to increased age-independent mortality, as well as an increased rate of ageing in females (Table 2).

Although selection regime did not have a statistically significant effect on the extent to which crowding reduced longevity (Table 1), our results suggest that the UC populations may, in fact, have a reduced sensitivity to adult crowding, as a result of having been subjected to high adult densities for over 50 generations (Figure 1). Possibly, a more severe bout of crowding would lead to differences among the selected lines that would be picked up as being statistically significant. This interpretation is supported by the finding that the UC populations are significantly less sensitive to adult crowding effects on fecundity and mortality during crowding than either the CU or the UU populations[41].

One very consistent finding of previous studies on environmental and genetic modifications of longevity has been the existence of a ubiquitous trade-off between female fecundity and longevity[2,3,17]. This trade-off has been interpreted in light of the so-called Y-model of resource allocation[6,10,42]. In this view, reproduction and survival are envisioned as being alternative candidates for the allocation of energy reserves by organisms, such that, all other things being equal, increased reproductive output comes at the expense of survival, and *vice versa.* A series of studies on *Drosophila* populations closely related to our CU, UC and UU lines, suggest that fecundity and longevity in these populations are, in fact, linked in this manner, based upon their common dependency on lipid as a resource[4,6,27,43]. Similarly, most environmental factors increasing or decreasing longevity in many species have been shown to do so by respectively reducing or enhancing fecundity. Since the reduction of female fecundity by adult crowding is a well-documented phenomenon in *Drosophila*[44], one might naively expect that adult crowding would, therefore, enhance longevity.

In fact, the observed reduction in longevity, by levels of adult crowding that are known to also depress fecundity in the UU, UC and CU populations[41], strongly suggests that the negative impact of adult crowding on longevity in our experiment is mediated by a reduction in the overall levels of stored energy reserves in the flies subjected to the crowded conditioning treatment. In this context, it is interesting to note that even 24 hours of adult crowding reduced subsequent starvation resistance in the B populations from which our experimental populations were derived[37]. Given the close relationship between starvation resistance and lipid content in *Drosophila*[4,6,8], this suggests that brief episodes of adult crowding may significantly deplete lipid reserves. However, there are also indications that reserves of glycogen, rather than lipid, may be more severely affected by adult crowding[37]. Evidently, further studies of the physiological consequences of adult crowding are needed to obtain a clearer picture of how the effects of crowding on adult fitness components are mediated by the underlying architecture of inter-related metabolic pathways.

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Neural network modelling of human electroencephalogram patterns

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A general regression neural network model with different processing elements (PE) fits the observed electroencephalogram signals (EEG) of the human brain during awake, sleep and rapid eye movement (REM) stages. The number of PEs required for simulation could be an indication of the complexity of the EEG pattern. About 6.5 times more PEs are required to simulate the signal during alert eyes open (β waves) than alert eyes closed (α waves) stage. Also, half the number of PEs are required to simulate sleep stage 4 when compared to simulating sleep stage 2. REM sleep requires more number of PEs to simulate than sleep stage 4. PEs required to simulate short duration 'petit mal' epileptic seizure less than those required to simulate β waves, but more than those required to simulate REM state. In most cases a correlation exists between the number of PEs and the fractal dimension.

THE neuron is the fundamental cellular unit of the nervous system and, especially the human brain. The central nervous system is a neural network of about 10^{11} neurons, all of them interconnected in the form of a dense matrix. The neocortex is under the influence of external stimuli as well as input from subcortical areas. The axon or the output path of a neuron splits up and connects to dendrites or input paths of other neurons through a junction known as a synapse. The electrical activity of the brain is routinely measured from the scalp and analysed for abnormalities. It is used in the search for sleep disorders.

Electroencephalogram (EEG) is related to the firing pattern of the neurons in the neighbourhood of the cerebral cortex. Cells continuously receive pulses, especially at dendrites, from other neighbouring neurons. The pulses are transmitted at synapses. Certain incoming pulses generate excitatory waves, while others generate inhibitory waves of electric current in the recipients. These dendritic currents are fed through the cell body to a region at the start of the axon. These currents enter the extracellular space after crossing the cell membrane. The cell calculates the overall strength of the currents, which is indicated by changes in voltage across the membrane, by adding all the excitatory and subtracting all the inhibitory currents. If the sum is above a threshold value then the neuron fires (i.e. an output from the neuron is generated). Although the activity of real neurons is very complex what is described above is an oversimplification of the process of synaptic integration.

The mechanism producing each EEG tracing sums up the currents initiated at the dendrites. The tracing shows the excitatory state of group of neurons rather than of individual neurons, because the extracellular space is criss-crossed by currents from thousands of cells[1]. EEG tracings of living beings generally oscillate and are very irregular, but nevertheless follow a pattern. They may have a high-frequency component superimposed on low-frequency signals. Recent progress in the theory of nonlinear dynamical systems has provided new methods for the study of EEG time series data. Global properties of the brain EEG patterns at various stages of activity and inactivity, are being analysed using the theory of chaos, Lyapunov exponent, auto-correlation function, power spectra and fractal dimension[2–4]. Activity of a small subset of neurons in the presence and absence of a synchronized oscillatory signal is modelled using differential equations and the results analysed using spatial auto-correlation function and correlation dimension[5].

Neural network computer programs try to simulate neuronal activity. In an artificial neural network, the unit analogous to the biological neuron is referred to as a processing element (PE)[6,7]. In spite of the fact that simple interconnected networks do not completely simulate the dynamic properties of real neurons, they are useful in gaining insight into universal principles of distributed systems. Also, a trained network has striking similarities to actual neurons and can suggest functional roles of neurons with input and output that are hard to grasp intuitively. Back propagation network has been used to process chaotic EEG signals[8].

A neural network consists of many processing elements joined together through connection weights which correspond to the synaptic strength of neural connections. Data to the network is presented at input layer and the response of the network to the given data is stored in the output layer. It is speculated that the training or learning of neural network model is similar to the way humans or animals are trained by reinforcement technique, where certain synapses that connect the neurons selectively get strengthened leading to increase in the gain.

We attempt here to simulate the different types of EEG patterns emanating from human brain during activity (eyes closed and open, epileptic seizure stages) and inactivity (sleep stages 2, 4 and REM stages) using a general regression neural network model.

Back propagation partially recurrent and cascade correlation neural network models have been used to simulate non-average single trial multi-channel EEG data produced during the side finger movement[9]. The cascade correlation neural network model was found to predict the obs signals well.

Neural network models have been used to train on ECG signals of patients with anterior and myocardial infarction and the network was later used for prediction of abnormalities[10]. Back propagation model has also been used for detecting spikes and seizures during epileptic fits[11], for simulating the oscillatory activity observed in the visual cortex[12], and olfactory system[13]. Very little work is reported on simulating the EEG brain signals during various sleep stages, eyes open and closed stages. A trained neural network model can help in identifying sleep disorders, changes in sleep pattern and variation in sleep patterns between individuals.

Two networks that are well suited for modelling, prediction and classification are back-propagation (BP) and general regression neural network (GRNN)[14]. BP network is a general purpose nonlinear regression technique which attempts to minimize global error. The main advantages of the GRNN over the BP network are (i) it can handle non-stationary or noisy data; (ii) it can be effectively used with sparse data. This network was proposed by Donald Specht of the Lockheed Palo Alto Research Laboratory.

Each PE in the network has several inputs and outputs. The output path of a PE is connected to the input path of another PE through connection weight. The input signal is multiplied by the weight before it is sent to the PE. All the weighted signals are summed up and fed as the input to the PE. This signal is transformed and sent to the output path of the PE. A neural network normally has one input, one output and at least one hidden layer and, there may be several PEs in each layer. During learning stage the weights are manipulated so that for a given set of input signals, the outputs estimated by the network match the actual values.

GRNN uses a statistical equation for calculating the conditional mean Y of a scalar random variable y given a measurement X of a vector random variable x. The conditional mean is statistically the most probable value for the random variable Y for given X. The vector random variables x and y correspond to the ensemble of inputs and outputs of the network. The equation for conditional mean requires knowledge of the joint probability density function (PDF) of the random variable x and y.

In GRNN, the PDFs are obtained from the training vectors using Parzen estimation. Parzen estimation is a non-parametric estimation procedure which approximates a density function by constructing it out from many PDFs and, it takes the form of a Gaussian distribution (bell-shaped curve). The parameter used in the exponential term of the Gaussian equation is estimated from the input vectors and their centre and, is given by the square root of the sum of square of the difference between the input vectors and the centre. It is also known as the Euclidean distance (D_t). This type of summation is called Euclidean summation and is suitable for most of the problems. The estimate for the conditional mean for the output vector is given by

$$Y(X)=\frac{\sum_{t=1}^{T}[Y^{(t)}\exp(-D_t^2/2\sigma^2)]}{\sum_{t=1}^{T}[\exp(-D_t^2/2\sigma^2)]},$$

where $\{[X^{(t)}, Y^{(t)}]: t=1, \ldots T\}$ is a set of training sample input/output pairs, D_t is the Euclidean distance between x and $X^{(t)}$ and N is the number of input modes of the network.

$$D_t=\sqrt{\sum_{i=1}^{N}(x_i-X_i^{(t)})^2}.$$

σ, the width parameter $= S/T^{E/N}$, $0 < E < 1$.

At the start of the learning cycle, the number of pattern (or hidden) PE can be set to the number of data vectors in the training file and later changed depending upon the network performance. Three parameters which control the learning process are the radius of influence, reset factor and time constant.

If the input vectors or training samples are large, the estimate for conditional mean becomes both time and memory-intensive. So the input vectors are grouped together (or clustered) and the average value of the groups are used in the calculations in order to decrease the simulation time. Any input vector is assigned to a nearest group if its distance to the centre of a previously established kernel is less than the radius of influence, otherwise a new kernel is established. A very small value for radius of influence will lead to a large number of clusters and hence increased computational time. Whereas, a large value will give fewer clusters and hence loss of sensitivity and possibly poor data fit.

PEs whose contributions in the summation are very small are termed as non-winning PEs. All the output weights from each of these non-winning PEs are multiplied

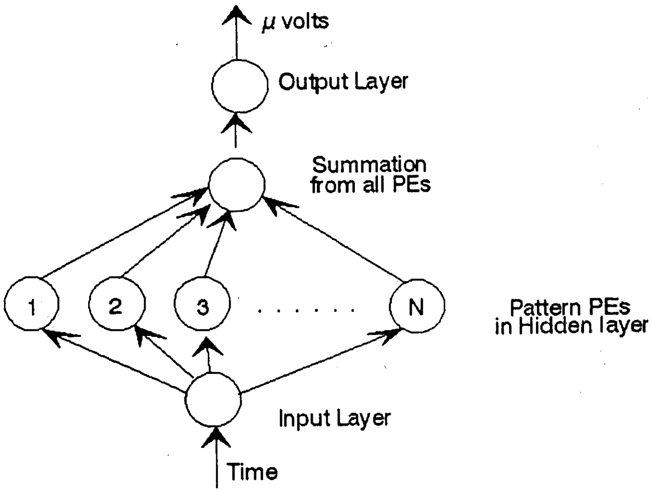

Figure 1. A general regression neural network for the EEG problem.

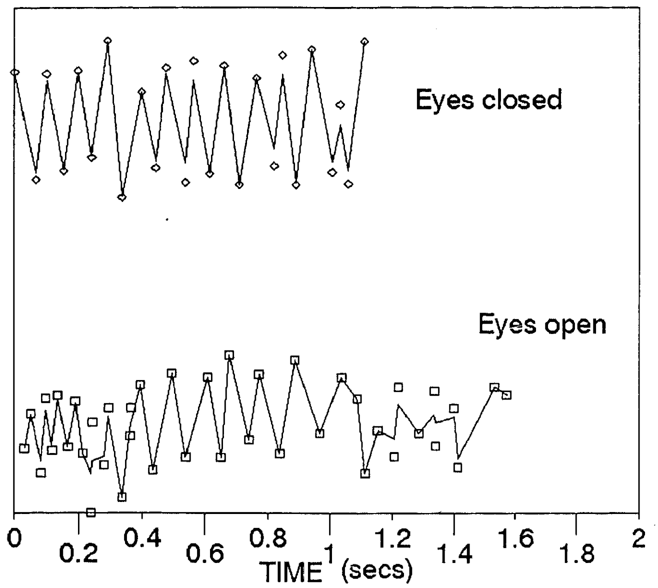

Figure 2. Comparison of human EEG data (points) and GRNN model simulation (continuous lines) during the stages of eyes open (9950 PEs) and eyes closed (1500 PEs). (Number of PEs required for simulation are given in brackets. *Y* axis is not to scale but presented arbitrarily to show the fit between the model and the simulation. *Y* axis ranges for eyes open and closed are 10 and 60 µV respectively.)

by a numeric term (called forgetting function), whose value decreases in an exponential fashion. The value of the time constant in the exponential term decides how fast the weights decay. When the weights become smaller than the ratio, reset factor/number of PEs, then the particular weight is set to zero. A large time constant could lead to slow decay of the weight from non-winning PE. The suggested value for radius of influence, reset factor and time constant are 0.05, 0.01 and 1000 respectively. In all the simulations about 10000 learning cycles were employed. Figure 1 shows a typical GRNN with a single input and single output, in this case time and µV respectively, and several pattern PEs in the hidden layer.

The EEG patterns were simulated by GRNN network. NeuralWorks Explorer (NeuralWare Inc., Pittsburgh, USA), a software running on a PC 386 equipped with a math coprocessor, was used for these studies[15]. The human EEG brainwave data were read from the figures given in references 3 and 4. The blown-up figures were scanned using a flat bed optical scanner, digitized and the data points were read into a computer program using a mouse and a cursor. The data points were uniformly spaced out in time. Time versus µV were read out from the digitized data. About 5000 data points were collected from each pattern signal, 80% of the data was used by the neural network model for fitting and the remaining 20% of the data was used for comparing model predictions with actual values.

The goodness-of-fit (root mean square error = RMSE) is obtained by calculating the square root of the mean of the sum-of-squares of the difference between model predictions and the actual data. Generally RMSE decreases as the PEs are increased. The simulations were performed by systematically increasing the number of PEs until the corresponding RMSE reaches a minimum value or remains unchanged. The PEs reported in the paper correspond to the lowest RMSE. In all the figures, points represent observed values and continuous lines are the neural network model predictions. In the figures only a few data points are shown for the sake of clarity. Also, data points near peaks and valleys are shown because maximum differences are generally expected between model predictions and data at these points.

An alert and active brain produces beta waves, which are high frequency low amplitude events (~ 10 µV) (ref. 4). As the eyes are closed, the relaxing individual produces alpha waves, with an average frequency of 10 cycles/s and amplitudes of the order of 60 µV. Alpha waves are well ordered and much more coherent than beta waves. Figure 2 shows the comparison of EEG data with simulation of both these waves with a GRNN network model. The alpha waves were simulated with 1500 PEs, whereas the beta waves were simulated with 9900 PEs. Both the simulations were carried out with 10,000 learning cycles and, radius of influence of 0.0001. A very low radius of influence indicates that no clustering of PEs was done. From the figure it could be noticed that the model is able to predict the observed behaviour reasonably well. Beta waves have fine structure, hence require more PEs (by an order of 6.5) to simulate than the well-ordered alpha waves. As the brain reaches a relaxed stage (alpha) from an alert one (beta), the number of neurons in an active state in the brain possibly decreases, which is very clearly seen from the number of PEs required for simulation.

In sleep the individual drifts in and out of four stages of sleep. Sleep stage 2 is a light stage, with slightly coherent waves of amplitude of 70 µV. Sleep stage 4, is a deep sleep stage, with amplitude of 120 µV and about 3–5 cycle/s. The four stages of sleep are followed by the rapid eye movement sleep (REM) in which he dreams. In the REM stage the cerebral activity reverts to a non-coherent state, with an amplitude of about 30 µV. All these three sleep stages are simulated with a GRNN network having 4000, 2000 and 2500 PEs respectively. Figure 3 compares the data with the simulation. As the sleep enters the deep stage 4 from stage 2, the number of PEs decreases by half, indicating that the number of neurons active in the brain also decreases, possibly by half. The number of PEs required to simulate REM sleep is more than that required to simulate sleep stage 4, but less than that required to simulate stage 2. The EEG brain wave data shown in Figures 2 and 3 were taken from reference 4.

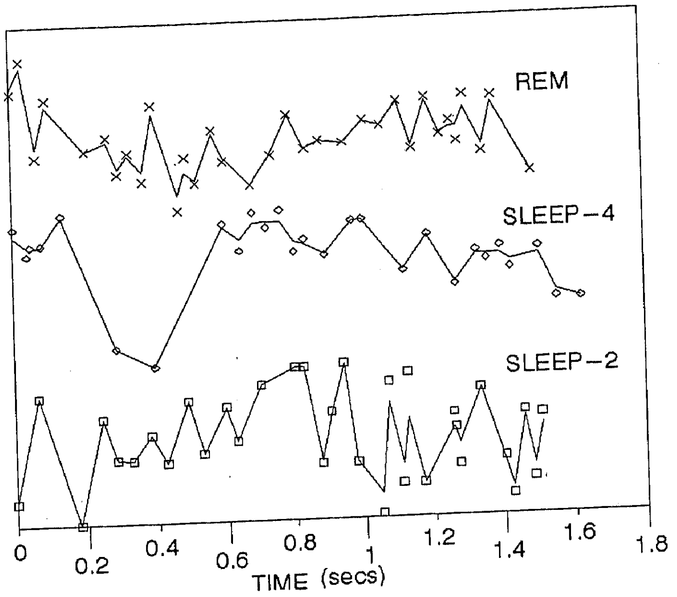

Figure 3. Comparison of human EEG data (points) and GRNN model simulation (continuous lines) during sleep stage 2 (4000 PEs), sleep stage 4 (2000 PEs) and REM sleep (2500 PEs). (Number of PEs required for simulation are given in brackets. *Y* axis is not to scale but presented arbitrarily to show the fit between the model and the simulation. *Y* axis ranges for sleep stages 2, 4 and REM are 70,120 and 30 μV respectively.)

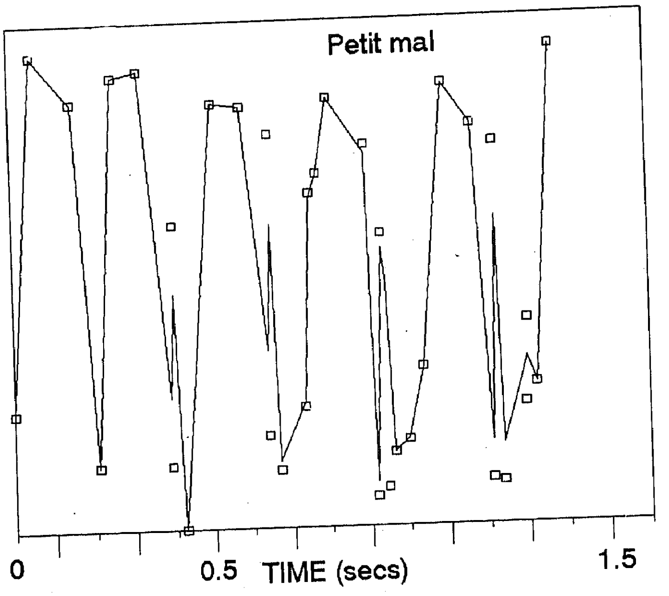

Figure 4. Comparison of human EEG data (points) and GRNN model simulation (continuous line) during petit mal seizure (5000 PEs). (Number of PEs required for simulation are given in brackets. *Y* axis range for petit mal seizure is 120 μV.)

There are several forms of epilepsy and, seizure of short duration (~5 s) is known as 'petit mal'[3]. This type generally invades the entire cerebral cortex and shows a bilateral symmetry between the two hemispheres. During the seizure the EEG activity switches to an apparent oscillating mode. Figure 4 compares the data and the simulation carried out with a GRNN network, of the EEG pattern of the epileptic stage with 5000 PEs. The model appears to simulate the epileptic waves fairly well. It appears that the number of neurons taking part during the seizure is more than the normal REM stage but less than the eyes open active stage. The EEG data of 'petit mal' was taken from reference 3.

Table 1. Comparison of human cerebral activity, number of PEs required in the GRNN neural network model for simulation and the fractal dimension of the EEG signals

Activity	Number of PEs required for simulation	Fractal dimension (from refs 2–4)
Eyes open	9950	9.7
Eyes closed	1500	6.6
Sleep stage 2	4000	5.0
Sleep stage 4	2000	4.05
REM	2500	8.2
Petit mal	5000	2.05

Table 1 lists the number of PEs required for simulating the EEG waves at various stages of brain activity. It also gives the fractal dimension, which is a measure of the minimum number of variables required to describe the signal[2–4]. Fractal dimension also measures the temporal coherence of the phenomena and follows qualitatively the degree of arousal. The fractal dimension decreases from the eyes open to the eyes closed stages, and so does the number of PEs required for simulating these two states. A similar trend in fractal dimension and number of PEs is observed in the case of sleep stages 2 and 4. Although the fractal dimension in the case of REM sleep is high, the number of PEs required for simulation is low. The fractal dimension of the EEG signals during the REM stage and epileptic seizure are 8.2 and 2.05 respectively, indicating a large reduction in the complexity of the signals. The neural network model studies indicate that the number of PEs required for both these stages are 2500 and 5000 respectively. The PEs required for simulating 'petit mal' seizure should have been much lower than this value. The RMSE in all cases is of the order of 0.05 to 0.07 (error margin of about 5 to 7%), indicating a good data fit.

This paper describes the modelling and simulation of the EEG patterns observed during the various stages of human activities. The simulations were carried out with general regression neural network model. The number of neurons required to simulate the EEG patterns and rhythms could be reasonably explained based on the brain activity. Most number of PEs are required to simulate beta waves observed during an active brain with eyes open. This is followed by 'petit mal' seizure. A correlation exists between number of PEs and fractal dimension in some cases. It should be borne in mind that the number of PEs required for simulating a particular activity estimated from modelling, cannot be equal to the number of neurons in the brain taking part in the activity. But, the ratio of PEs estimated by modelling for two activities may be an indication of the ratio of active neurons in the brain during two activities. Although another neural network model, namely back propagation model, was not able to fit these EEG patterns, other neural network models could also be attempted.

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Response of IgG sub-classes to diethylcarbamazine therapy in bancroftian filarial patients

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The response of IgG subclasses to diethylcarbamazine (DEC) treatment was studied in bancroftian filariasis patients. On the basis of clinical signs and parasitological examination, a total of 22 patients were categorized into asymptomatic microfilaraemics (AS-Mfmic; $n = 12$) and symptomatic amicrofilaraemics (S-AMfmic; $n = 10$). The subjects were treated with DEC (300 mg/day) for 21 days. Before treatment, AS-Mfmic cases showed higher levels of IgG_1 and IgG_4 than the S-AMfmics whereas IgG_2 was higher in S-AMfmics than in AS-Mfmics. DEC caused more than 90% reduction in microfilaraemia by day 30 since the start of treatment in AS-Mfmics, while S-AMfmics remained amicrofilaraemic throughout the study period. In AS-Mfmics, DEC treatment enhanced IgG_4 and decreased IgG_1 levels while IgG_2 and IgG_3 remained unaffected. In S-AMfmics, DEC treatment caused decrease in IgG_1, IgG_3 and IgG_4, while IgG_2 level remained unchanged. We report that DEC therapy brings about changes in specific IgG_1 and IgG_4 in AS-Mfmics and IgG_1, IgG_3 and IgG_4 in S-AMfmics.

FILARIAL parasite initiates immune response in its host at both cellular and humoral levels. Clinical expression of filariasis, therefore, reflects not only the duration and intensity of infection but also the degree and character of different types of immunologic responses. Asymptomatic microfilaria (mf) carriers have depressed antibody- and cell-mediated immune responses while acute manifestations and chronicity are associated respectively with an intermediate and hyper-immune response[1–4].

The major immunoglobulins involved in the antifilarial antibody responses in human host are IgG, IgM, and IgE[3,5,6]. IgG is the major immunoglobulin detectable in all categories of filarial subjects and the clinical severity of the infection is directly related to this isotype[7]. Recent studies have also shown that different categories of filarial subjects have different IgG subclass profiles. Specific IgG_1 and IgG_3 are predominant in chronic lymphatic filariasis whereas IgG_4 is elevated in mf carriers and tropical pulmonary eosinophilia cases[8,9]. As IgG_4 was suggested to indicate the presence of parasites[8,10], Wamae *et al.*[11] used it as an indicator of adulticidal efficacy of diethylcarbamazine (DEC) or ivermectin in microfilaraemic (bancroftian) human subjects. However, whether DEC can also bring about alteration in other subclass responses in bancroftian filarial patients is not known. We report here the response of IgG subclasses to DEC treatment (shortly after cessation of the treatment) in symptomatic amicrofilaraemic and asymptomatic microfilaraemic bancroftian patients.

Patients reporting to the outdoor clinic of King George's Medical College, Lucknow for treatment of various ailments, were examined for filariasis. The patients were from Lucknow and its adjoining areas which are known to be endemic to bancroftian filariasis. A total of 22 patients diagnosed clinically and parasitologically positive for filariasis were included in the study. Of these, 12 subjects were asymptomatic with microfilaraemia (AS-Mfmic) while 10 had symptoms of 3–4 years duration (elephantiasis of legs and/or hydrocele) but amicrofilaraemic (S-AMfmic). Microfilariae (mf) in venous blood (1 ml; collected between 9 and 11 pm) were assessed using the membrane filtration technique[12]. For the assay of IgG and its subclasses, serum was separated from blood and stored at –20°C till use.

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*For correspondence.

All the AS-Mfmic and S-AMfmic subjects received diethylcarbamazine (DEC) at 300 mg/day for 21 days. Venous blood was collected before therapy and one week after the last dose. Treated patients were examined daily during and after the therapy for any adverse reactions.

Brugia malayi adult worms were harvested from intraperitoneal cavity of jirds infected intraperitoneally with *B. malayi* L_3. Soluble somatic extract of the worms was prepared as described by Lammie *et al.*[13] and used as the antigen source. The protein content of the extract was determined by the method of Lowry *et al.*[14].

Circulating antifilarial IgG subclasses were assessed by ELISA as described by Hussain *et al.*[15], with some modifications. Briefly, polystyrene plates were coated overnight at 4°C with the antigen (10 µg/ml) in carbonate buffer. Unsaturated sites of the surface were blocked with 1% gelatin in phosphate buffered saline (G-PBS) for 1 h at 37°C, and the plates were incubated with sera diluted (1:25) in G-PBS containing 0.01% Tween-20 (G-PBS-T) for 90 min at 37°C. The plates were washed with PBS-T and incubated with optimally diluted mouse monoclonal anti-human IgG_1 (1:7,500), IgG_2 (1:5000), IgG_3 (1:5000) and IgG_4 (1:7500) for 90 min at 37°C. The plates were again washed and incubated for 90 min at 37°C with 1:1000 dilution of anti-mouse IgG-peroxidase conjugate. The plates were washed and incubated in the substrate medium consisting of 0.08% each of *o*-phenylenediamine and H_2O_2 in citrate buffer (pH 5.0). After stopping the reaction with 2.5N H_2SO_4, the optical density was read at 492 nm using an automated ELISA reader (Multiscan). All the antibodies and conjugates were from Sigma Chemical Co., St. Louis.

The values of the IgG subclasses in the two categories of the patients were compared by Student's *t* test. Paired *t* test was applied for the analysis of effects of DEC treatment on the responses. Differences were considered significant if $p \leq 0.05$.

The concentration of specific IgG subclasses (IgG_1, IgG_2, IgG_3 and IgG_4) in sera of AS-Mfmic and S-AMfmic cases before and after DEC treatment is shown in Figure 1.

Amongst the four subclasses of IgG, the levels of IgG_4 alone and IgG_2 and IgG_4 were significantly elevated ($p < 0.01$) respectively in AS-Mfmic and S-AMfmic cases. When the values of the 4 IgG sub-classes were compared between the two groups, the responses of IgG_1 and IgG_4 were significantly higher ($p < 0.01$) in AS-Mfmic while IgG_2 was greater in S-AMfmic cases ($p < 0.05$).

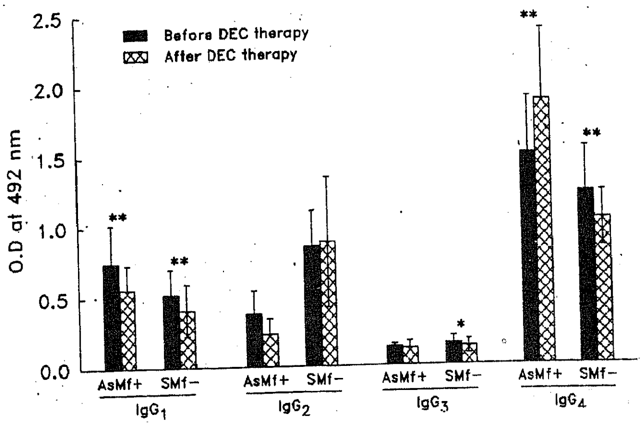

Figure 1. IgG subclasses in asymptomatic microfilaraemics and symptomatic amicrfilaraemics before and after DEC therapy (*$p < 0.05$; **$p < 0.01$).

By day 30 post-DEC treatment, there was more than 90% reduction (88.29–98.33%) in microfilaraemia in AS-Mfmic cases while the S-AMfmic patients remained amicrofilaraemic (data not shown). There was no amelioration of manifestations in S-AMfmic cases nor any appearance/development of tenderness in any part of the body in AS-Mfmic cases during the course of treatment.

In AS-Mfmic subjects, DEC treatment decreased the antifilarial IgG_1 response ($p < 0.01$) but increased specific IgG_4 response ($p < 0.01$). IgG_2 and IgG_3 responses were not affected by DEC therapy.

In S-AMfmic cases, DEC treatment resulted in decrease in IgG_1 ($p < 0.01$) and IgG_4 ($p < 0.01$), and IgG_3 ($p < 0.05$) whereas IgG_2 remained unaffected.

Responses of IgG subclasses (IgG_1, IgG_2, IgG_3 and IgG_4) have been reported for chronic helminthic diseases, namely, filariasis, schistosomiasis and cysticercosis; IgG_4 was found to be the most dominant subclass in these diseases[16–18] though it accounts for only 3–4% of the total IgG in normal human serum[19]. The present study revealed that in the untreated subjects the concentration of subclass IgG_4 was maximum in AS-Mfmics while IgG_2 and IgG_4 concentrations were raised in S-AMfmic subjects. Inter-category comparison showed that IgG_1 and IgG_4 were higher in AS-Mfmics than in S-AMfmics, whereas IgG_2 was higher in S-AMfmics than in AS-Mfmics.

A significantly high level of IgG_1, as found in AS-Mfmics in this study, was also reported by Mak *et al.*[20] in brugian microfilaraemic patients but not in bancroftian microfilaraemics. S-AMfmic cases had high IgG$_2$ compared to AS-Mfmic patients which supports the findings of Rahmah *et al.*[21] in malayan filariasis. These authors even suggested that IgG$_2$ may be used as diagnostic tool for *B. malayi*-induced chronic elephantiasis. On the other hand, Ottesen *et al.*[8] reported elevated antifilarial IgG$_1$ and IgG$_4$ but not IgG$_2$ in patients with chronic elephantiasis due to *W. bancrofti* infection.

IgG$_3$ was lower than other subclasses in both the categories of subjects. This is in contrast to the findings of Hussain *et al.*[15] who found low IgG$_3$ levels only in AS-Mfmic bancroftian filarial cases. AS-Mfmic brugian cases, on the other hand show elevated levels of IgG$_3$ (ref. 20).

We found elevated IgG$_4$ levels in both AS-Mfmics and S-AMfmic patients. Ottesen *et al.*[8], Hussain *et al.*[15], Lal and Ottesen[9] and Kwan-Lim *et al.*[22] reported predominance of IgG$_4$ antibody response not only in mf (*W. bancrofti*) carriers but also in tropical pulmonary eosinophilia cases.

Significant variations have been reported in the levels of IgG subclasses in filarial subjects. Although no explanation is forthcoming for this disparity, the strain of parasite prevalent, the immune status of population studied and many other associated factors might be responsible for these variations.

DEC treatment resulted in significant decrease in IgG$_1$ levels in AS-Mfmics and IgG$_1$, IgG$_3$ and IgG$_4$ levels in S-AMfmics. While the present studies were in progress, Atmadja *et al.*[23] reported a decrease in IgG$_1$ and IgG$_4$ levels 12 months after DEC therapy in *malayan* microfilaraemic and elephantiasis patients. However, they reported a fall in IgG$_2$ as well as IgG$_3$ in elephantiasis patients while our present study showed no change in IgG$_2$ response in S-AMfmics by day 30 since the initiation of treatment. In view of the known role of IgG$_1$ and IgG$_3$ in hypersensitivity/allergic (Type III) reactions[24] which lead to tissue damage and the recent report of Casley-Smith *et al.*[25] that DEC reduced lymphoedema in chronic symptomatics, these two IgG sub-classes may be involved in the responses to DEC treatment in symptomatic amicrofilaraemic bancroftian subjects. In AS-Mfmics on the other hand, IgG$_1$ is evidently involved in the responses to DEC treatment. It is interesting to note that recently, using lymphoscintigraphy, lymphatic tissue pathology was demonstrated in asymptomatic microfilaraemics also[26]. If IgG$_1$ is involved in tissue pathology seen in asymptomatic microfilaraemics, as suggested by the above reports, our results indicate that this subclass in combination with IgG$_4$ (discussed below) may be useful as a convenient tool for assessing the response of microfilaraemics to DEC therapy.

In AS-Mfmic subjects, IgG$_4$ levels were increased by DEC treatment by day 30 since the initiation of therapy. This confirms the recent finding of Wamae *et al.*[11] who found increased IgG$_4$ levels at day 30 since start of a twelve-day course of DEC therapy in bancroftian microfilaraemics which decreased significantly to below pretreatment levels by day 180. As suggested by these authors, the increase in IgG$_4$ levels in our microfilaraemic subjects is related to sudden release of microfilarial antigen(s). The significant reduction (more than 90%) in microfilaraemia found in our subjects supports this suggestion.

In conclusion, the DEC therapy brings about changes in specific IgG$_1$ and IgG$_4$ in bancroftian AS-Mfmics and in IgG$_1$, IgG$_3$ and IgG$_4$ sub-classes in S-AMfmics. Assessment of IgG subclass levels in AS-Mfmics and S-AMfmics may possibly be used as a convenient tool for monitoring the infection and especially the clinical status of the disease following antifilarial therapy.

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Synthesis and antitumour activity of new derivatives of podophyllotoxin

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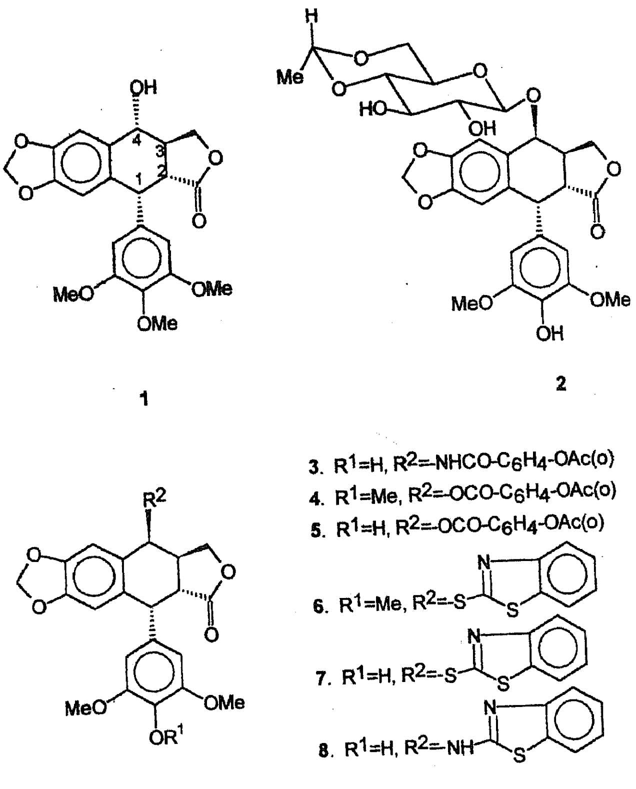
A series of new podophyllotoxin derivatives 3–8 have been synthesized and evaluated for their antitumour activity *in vitro*. Compounds 3 and 8 exhibited comparable or superior activity to clinically used etoposide (VP-16, 2) in their inhibition of human stomach carcinoma SGC-7901, lung cancer A 549, and mouse leukemia P388 cells.

SEMISYNTHETIC analogues of the naturally-occurring podophyllotoxin (**1**) have drawn much renewed interest in recent years as a result of the development of etoposide (VP-16, **2**) and teniposide (VM-26) as anticancer drugs[1,2]. It is believed that analogues of 4′-demethylepipodophyllotoxin exert their antitumour activity through stabilization of a cleavable complex between DNA and type II DNA topoisomerase. This leads ultimately to inhibition of DNA catenation activity and produces single and double strand breaks[3,4].

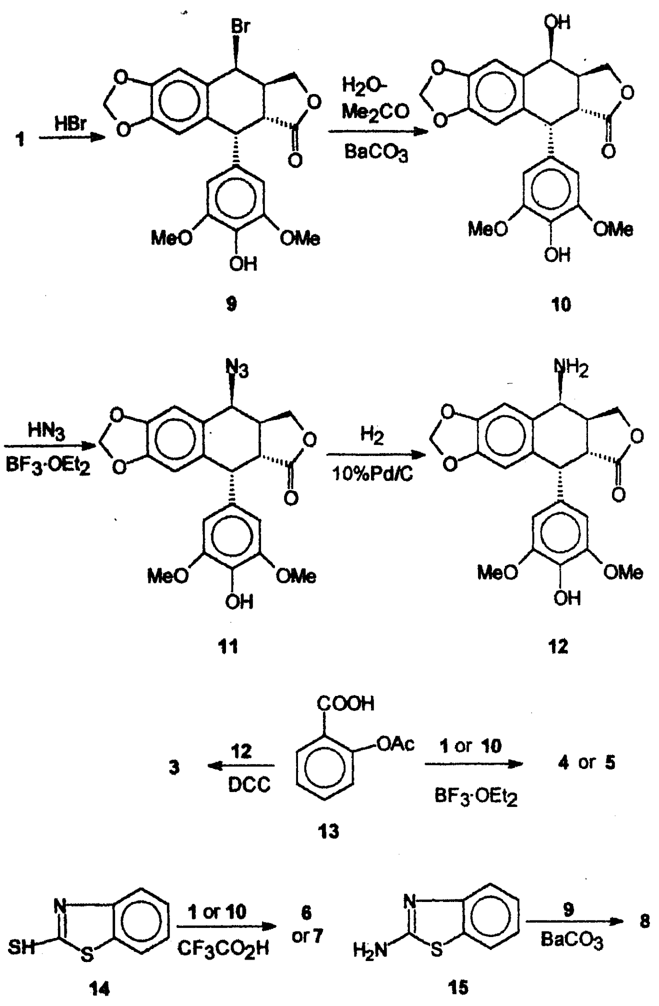
In our previous studies[5–8], we found that substitution of the glycosidic moiety in **2** by a configurationally similar nitrogen-containing group led to some compounds which have comparable or superior antitumour activity to **2**. The results suggested that the β-anomeric configuration at C-4 was indispensable for the antitumour activity. Changes in the 4β-glycosyl group are also of interest for simplified structure which might retain the activity of **2**, and be accessible to practical industrialization. Here we wish to present the synthesis of a series of new analogues of epipodophyllotoxin **3–8** and their biological activities *in vitro*.


OH
4
3
1
2
O
MeO
OMe
1
H
Me
HO
OH
2
R2
OR1
3. R1=H, R2=-NHCO-C6H4-OAc(o)
4. R1=Me, R2=-OCO-C6H4-OAc(o)
5. R1=H, R2=-OCO-C6H4-OAc(o)
6. R1=Me, R2=-S
7. R1=H, R2=-S
8. R1=H, R2=-NH
N
S

Scheme 1.

The synthesis of target compounds started from **1** as shown in Scheme 2. 4β-bromo-4′-demethyl-4-deoxypodophyllotoxin (**9**) and 4′-demethylepipodophyllotoxin (**10**) were prepared from **1** by our previous procedure[5,9]. **10** was treated with HN_3 to yield 4β-azido-4′-demethyl-4-deoxypodophyllotoxin (**11**) as the major product, which was accompanied by the C-4 isomer product, 4α-azido-4′-demethyl-4-deoxypodophyllotoxin, **11** can be purified by crystallization. Further reduction of **11** led to 4β-amino-4′-demythyl-4-deoxypodophyllotoxin (**12**). Condensation of **12** with aromatic acid **13** in the presence of DCC gave compound **3**. Compounds **4** and **5** were synthesized by the reaction of **13** with **1** and **10**, respectively. Thio-etherification of **1** and **10** with 2-mercaptobenzothiazole (**14**) yielded compounds **6** and **7**, respectively. Compound **8** was synthesized by direct substitution of 2-amino-benzothiazole (**15**) with 4β-bromo-4′-demethyl-4-deoxypodophyllotoxin (**9**).

All new target compounds were characterized by m.p., ^{1}H NMR, MS and IR spectral analysis, as well as elemental analysis. The assignment of the configuration at C-4 for compounds **3–8**, **11** and **12** was based on the difference of $J_{3,4}$ coupling constants. The C-4β-substituted compounds **3–8**, **11** and **12**, have a $J_{3,4} \approx 4.0$ Hz as seen in **2** and **10** (ref. 10), due to a *cis*



Scheme 2.

Table 1. Cytotoxicity of compounds 3–8

Compound	ID50 (μM)		
	P388	SGC-7901	A549
VP-16(2)	0.012	0.32	0.44
3	0.0082	0.38	0.20
4	0.65	0.86	0.90
5	0.32	0.60	0.84
6	0.20	>10	0.40
7	0.26	>10	2.3
8	0.020	0.40	0.24

relationship between H-3 and H-4. The C-4α-substituted derivatives, however, like **1**, have a $J_{3,4} \geq 10.0$ Hz as H-3 is *trans* to H-4 (ref. 10).

We have tested the inhibitory activities of compounds **3–8** against human stomach carcinoma SGC-7901, lung cancer A549 and mouse lymphocytic leukemia P388 cells *in vitro*. As illustrated in Table 1, compounds **3** and **8** are as active or more active than VP-16 (**2**) in their inhibition of P388 and SGC-7901 cells, while compounds **3**, **6** and **8** are more potent than VP-16 in their inhibition of A 549 cells. Therefore, compounds **3** and **8** have comparable or superior antitumour activity to VP-16 (**2**). These results demonstrate the possibility of considerable simplification in the sugar structure of VP-16 and suggest further elaboration of 4β-nitrogen-containing substituent to optimize the structure of this class of anticancer compounds. Further study for antitumour activity of compounds **3** and **8** is in progress.

All melting points were taken on Yanaco melting point apparatus and uncorrected IR spectra were obtained on a Nicolet-5DX spectrophotometer, and ^{1}H NMR spectra were obtained by using either a Broker AM-400 or JMS-FX-90Q NMR spectrometer, all chemical shifts were reported in ppm from TMS. Elemental analyses were determined on a Carlo Elba 1106 instrument. Mass spectral analyses were taken on a V.G. ZAB-HS instrument at 70 eV with a direct inlet system.

4β-Azido-4′-demethyl-4-deoxypodophyllotoxin (**11**): To a solution of a mixture of 5.0 g (12.5 mmol) of **10** in 175 ml of anhydrous CH_2Cl_2 and 13 mmol HN_3 in benzene (10 ml) was added dropwise $BF_3.OEt_2$ (2.25 ml) at −16°C. The mixture was stirred at −16°C for 1 h. After being quenched with pyridine (2.25 ml), the solution was washed with water and dried over Na_2SO_4. Evaporation of the solvent gave a solid, which was checked by TLC and NMR analyses to be a mixture of 4α- and 4β-isomers (ca. 1:8). Crystallization from acetone/methanol provided 4β-isomer (**11**). Yield 91%, m.p. 213–214°C; MS m/z $[M]^+$ 425; IR (KBr) 3406 (OH), 2096 (azide), 1762 (lactone), 1610, 1520 and 1484 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.82 (s, 1H, H-5), 6.58 (s, 1H, H-8), 6.27 (s, 2H, H-2′, 6′), 6.01 (d, 2H, OCH_2O), 5.47 (s, 1H, 4′-OH), 4.81 (d, $J = 4.0$ Hz, 1H, H-4), 4.63 (d, $J = 4.4$ Hz, 1H, H-1), 4.32 (d, $J = 9.5$ Hz, 2H, H-11), 3.78 (s, 6H, 3′, 5′-OCH_3), 3.17 (dd, $J = 4.4$, 12.6 Hz, 1H, H-2), 2.93 (m, 1H, H-3); Anal. calcd for $C_{21}H_{19}N_3O_7$, C59.29, H4.50, N9.88; found C59.15, H4.51, N9.77.

4β-Amino-4′-demethyl-4-deoxypodophyllotoxin (**12**): To a solution of 5.0 g (11 mmol) of **11** of 100 ml of EtOAc was added 1.0 g of 10% palladium on carbon. This mixture was shaken under 40 psi of H_2 for 16 h. The reaction mixture was filtered through celite and the filtrate evaporated *in vacuo*. Crystallization from methanol gave 3.1 g of pure **12**, yield 68%, m.p. 212–214°C; MS m/z $[M]^+$ 399; IR (KBr) 3360 (OH), 3290 (NH_2), 1745 (lactone), 1610, 1525 and 1485 (aromatic C=C) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.81 (s, 1H, H-5), 6.49 (s, 1H, H-8), 6.30 (s, 2H, H-2′, 6′), 5.96 (d, 2H, OCH_2O), 5.06 (s, 1H, 4′-OH), 4.55 (d, $J = 5.2$ Hz, 1H, H-1), 4.28 (d, $J = 9.5$ Hz, 2H, H-11), 4.20 (d, $J = 4.1$ Hz, 1H, H-4), 3.77 (s, 6H, 3′,5′-OCH_3), 3.28 (dd, $J = 5.2$, 14 Hz, 1H, H-2), 2.85 (m, 1H, H-3) ppm; Anal.

calcd for $C_{21}H_{21}O_7N{\cdot}H_2O$, C60.43, H5.55, N3.36, found C60.52, H5.54, N3.38.

Compound **3**: 0.20 g (0.5 mmol) of **12** was dissolved in 20 ml of dried CH_2Cl_2, 0.09 g (0.5 mmol) of acetyl salicylic acid (**13**) and 0.10 g (0.5 mmol) of DCC were added, respectively. The reaction mixture was stirred at room temperature for 5 h. Two drops of acetic acid was added. The reaction mixture was filtered. The filtrate was washed with saturated solution of $NaHCO_3$, 10% hydrochloric acid and water, respectively, dried over anhydrous Na_2SO_4, and chromatographed through silica gel using $CHCl_3$-Me_2CO (6:1) as eluent. The pure product **3** (0.14 g, 5%) was crystallized from $CHCl_3$, m.p. 214–216°C; MS m/z $[M]^+$ 561; IR (KBr) 3400–3500 (OH, NH), 1765 (lactone), 1710 (ester), 1690 (CONH), 1610, 1510 and 1480 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.14–7.78 (m, 4H, H-3″, 4″, 5″, 6″), 6.92 (s, 1H, H-5), 6.56 (s, 1H, H-8), 6.32 (s, 2H, H-2′, 6′), 5.99 (d, 2H, OCH_2O), 5.42 (s, 1H, 4′-OH), 4.64 (d, $J = 4.0$ Hz, 1H, H-4), 4.50 (d, $J = 5.0$ Hz, 1H, H-1), 4.20 (d, $J = 9.8$ Hz, 2H, H-11), 3.60 (s, 6H, 3′, 5′-OCH_3), 2.96 (m, 2H, H-2, 3), 2.21 (s, 3H, 2″-$OCOCH_3$) ppm; Anal. calcd for $C_{30}H_{27}NO_{10}$, C64.17, H4.81, N2.50, found C63.81, H4.81, N2.38.

Compound **4**: To a solution of 1.5 g (3.6 mmol) of **1** and 0.65 g (3.6 mmol) of **13** in 50 ml of dried $CHCl_3$ was added dropwise $BF_3{\cdot}OEt_2$ (0.8 ml) at –16°C. The mixture was stirred at –16°C for 1 h. After quenched with pyridine (0.8 ml), the mixture was washed with water, dried over anhydrous Na_2SO_4, chromatographed on a silica gel column using $CHCl_3$-Me_2CO (7:1) as eluent, and crystallized from CH_2Cl_2 to give 1.0 g of pure product **4**. Yield 50%, m.p. 111–113°C; MS m/z $[M]^+$ 576; IR (KBr) 1778 (lactone), 1700 (ester, 1624, 1585 and 1487 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.21–7.91 (m, 4H, H-3″, 4″, 5″, 6″), 6.91 (s, 1H, H-5), 6.60 (s, 1H, H-8), 6.35 (s, 2H, H-2′, 6′), 6.01 (d, 2H, OCH_2O), 4.75 (d, $J = 4.2$ Hz, 1H, H-4), 4.42 (d, $J = 5.0$ Hz, 1H, H-1), 4.20 (m, 2H, H-11), 3.82 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.15 (m, 2H, H-2, 3), 2.20 (s, 3H, 2″-$OCOCH_3$) ppm; Anal. calcd for $C_{31}H_{28}O_{11}$, C64.58, H4.86, found C64.50, H4.77.

Compound **5**: **5** was synthesized from **10** by the similar procedure for the preparation of **4** from **1**. Yield 46%, m.p. 202–203°C; MS m/z $[M]^+$ 562; IR (KBr) 3367 (OH), 1778 (lactone), 1712 (ester), 1624, 1568 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.90 (m, 1H, H-6″), 7.60 (m, 1H, H-5″), 7.35 (m, 1H, H-4″), 7.19 (m, 1H, H-3″), 6.91 (s, 1H, H-5), 6.61 (s, 1H, H-8), 6.33 (s, 2H, H-2′, 6′), 6.01 (d, 2H, OCH_2O), 5.46 (s, 1H, 4′-OH), 4.74 (d, $J = 4.3$ Hz, 1H, H-4), 4.40 (d, $J = 5.1$ Hz, 1H, H-1), 4.15 (m, 2H, H-11), 3.61 (s, 6H, 3′, 5′-OCH_3), 3.10 (m, 2H, H-2, 3), 2.16 (s, 3H, 2″-$OCOCH_3$) ppm, Anal. calcd for $C_{31}H_{26}O_{11}$, C64.06, H4.63, found C64.27, H4.78.

Compound **6**: To a stirred mixture of 1.5 g (3.6 mmol) of **1** and 0.61 g (3.6 mmol) of 2-mercaptobenzothiazole (**14**) was added dropwise 19 ml of F_3CCOOH at –10°C. The reaction solution was stirred at room temperature for 3 h. Removal of the solvent yielded a residue, which was chromatographed on a silica gel column using $CHCl_3$-Me_2CO (7:1) as eluent, and crystallized from ethanol to obtain 0.85 g of **6**, yield 42%, m.p. 121–123°C, MS m/z $[M]^+$ 563; IR (KBr) 1772 (lactone), 1612, 1540 and 1490 (aromatic C=C) cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 7.29–7.64 (m, 4H, H-4″, 5″, 6″, 7″), 7.00 (s, 1H, H-5), 6.58 (s, 1H, H-8), 6.38 (s, 2H, H-2′, 6′), 6.00 (d, 2H, OCH_2O), 4.67 (d, $J = 4.2$ Hz, 1H, H-4), 4.40 (d, $J = 5.2$ Hz, 1H, H-1), 4.17 (d, $J = 9.5$ Hz, 2H, H-11), 3.82 (s, 3H, 4′-OCH_3), 3.75 (s, 6H, 3′, 5′-OCH_3), 3.23 (m, 2H, H-2, 3) ppm; Anal. calcd for $C_{29}H_{25}NO_7S_2$, C61.81, H4.44, N2.49, found C61.58, H4.37, N2.47.

Compound **7**: It was obtained from **10** by the similar procedure for the preparation of **6** from **1**. Yield 61%, m.p. 207–209°C; MS m/z $[M]^+$ 549; IR (KBr) 3400 (OH), 1778 (lactone), 1620, 1571 and 1485 (aromatic C=C) cm^{-1}; ^{1}HNMR ($CDCl_3$) δ 7.28–7.83 (m, 4H, H-4″, 5″, 6″, 7″), 7.02 (s, 1H, H-5), 6.52 (s, 1H, H-8), 6.37 (s, 2H, H-2′, 6′), 5.99 (d, 2H, OCH_2O), 5.47 (s, 1H, 4′-OH), 4.66 (d, $J = 4.2$ Hz, 1H, H-4), 4.48 (d, $J = 5.2$ Hz, 1H, H-1), 4.22 (d, $J = 10$ Hz, 2H, H-11), 3.63 (s, 6H, 3′,5′-OCH_3), 3.20 (m, 2H, H-2, 3) ppm; Anal. calcd for $C_{28}H_{23}NO_7S_2$, C61.20, H4.17, N2.55, found C60.99, H4.15, N2.35.

Compound **8**: A mixture of 0.47 g (1 mmol) of **9**, 0.25 g (1.7 mmol) of 2-aminobenzothiazole (**15**) and 0.23 g (1.1 mmol) of $BaCO_3$ in 10 ml of $ClCH_2CH_2Cl$ was stirred at room temperature for 24 h. The reaction mixture was filtered, diluted with ethylacetate, washed with water, dried over anhydrous magnesium sulphate, and purified via silica gel column chromatography using $CHCl_3$-Me_2CO (7:1) as eluent. Crystallization from ethyl acetate provided 0.26 g of **8**, yield 49%, m.p. 218–220°C; MS m/z $[M]^+$ 532; IR (KBr) 3550–3412 (OH, NH), 1774 (lactone), 1600, 1530 and 1487 (aromatic C=C) cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.54–7.16 (m, 4H, H-4″, 5″, 6″, 7″), 6.91 (s, 1H, H-5), 6.54 (s, 1H, H-8), 6.36 (s, 2H, H-2′, 6′), 5.98 (d, 2H, OCH_2O), 5.44 (s, 1H, 4′-OH), 5.20 (br, 1H, NH), 4.59 (d, $J = 4.0$ Hz, 1H, H-4), 4.40 (d, $J = 5.0$ Hz, 1H, H-1), 4.18 (d, $J = 9.5$ Hz, 2H, H-11), 3.80 (s, 6H, 3′, 5′-OCH_3), 3.04 (m, 2H, H-2, 3) ppm; Anal. calcd for $C_{28}H_{24}N_2O_7S$, C63.16, H4.51, N5.26, found C62.98, H4.56, N5.17.

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High frequency somatic embryogenesis and efficient plant regeneration from hypocotyl explants of groundnut (*Arachis hypogaea* L.)

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Embryogenic calli were obtained from hypocotyl explants of groundnut (*Arachis hypogaea* L.) cultured on a medium containing different concentrations of 2,4-D or NAA in combination with 0.5 mg/l BAP. Type of auxin, concentration and genotypes influenced somatic embryogenesis. 2,4-D was found to be the best somatic embryo inductor. As the auxin level increased beyond 20 mg/l in induction medium, both per cent as well as number of embryos were decreased. 2,4-D (20 mg/l) was found to be better than NAA for the induction of globular and heart-shaped somatic embryos. Somatic embryos developed from these calli following transfer to a medium supplemented with 0.5 mg/l 2,4-D and 2.0 mg/l BAP was found to be best for embryo maturation. The well-formed embryos germinated into plantlets on MS medium supplemented with BAP (0.5–2.0 mg/l) and NAA (0.25 mg/l). The regenerated plants were transferred to soil and grown to maturity. Hardened plantlets produced normal flowers and set viable seeds. The ability of this regeneration system to produce embryos exponentially offers potential for development of new gene transfer technology and application to synseed technology.

GROUNDNUT (*Arachis hypogaea* L.) is an important grain legume and its improvement programmes include development of varieties resistant to diseases like tikka (*Cercospora arachidicola* and *Cercosporidium personatum*), rust (*Puccinia arachidis*) and pest (red hairy caterpillar). An efficient regeneration protocol either by organogenesis or somatic embryogenesis is a major prerequisite for the application of gene transfer methods for crop improvement. Regeneration by organogenesis from various explants, viz. leaves, cotyledons, cotyledonary node, hypocotyl, epicotyl, zygotic embryos has been reported[1–6]. But regeneration frequency was low and plants were rarely established. A number of recent reports describe somatic embryogenesis in peanut using a variety of different explants, including leaves[7,8], immature cotyledons[9,10] and immature embryo axes[11,12]. In spite of these studies, efficient protocol for regeneration of plantlets via somatic embryogenesis is lacking[13]. This study describes the successful induction of somatic embryogenesis from hypocotyls and their subsequent development into plants from two important commercial cultivars.

Two popular cultivars of groundnut (*Arachis hypogaea* L. cvs VRI-2 and TMV-7) were used in this investigation. Seeds were removed from the pods and immersed in 100 ml of sterile distilled water with 5 drops of Tween 80 for 5 min. Surface sterilization with 0.1% (W/V) aqueous mercuric chloride for 7–10 min was followed by 5 rinses in sterile distilled water. Seeds were germinated on MS[14] basal medium in culture tubes and kept under dark for germination at 24 ± 2°C. Hypocotyl explants of 7-day-old seedlings were used as the sources of explants.

Eight to ten hypocotyl explants (0.5–1.0 cm length) were cultured in 250 ml conical flasks. The induction medium consisted of MS salts, B5 vitamins[15], 40 g/l sucrose with different concentrations of 2,4-D or NAA (1.0, 5.0, 10, 15, 20, 25 and 30 mg/l) in combination with BAP (0.5 mg/l). The pH of the medium was adjusted to 5.8, 0.5% (W/V) agar was added, and autoclaving was done at 121°C for 15 min. The cultures were incubated for four to six weeks at 24 ± 2°C under cool-white fluorescent light at 40 $\mu E\ m^{-2}\ s^{-1}$ with a 16 h photoperiod. The per cent of embryogenesis was calculated as follows: (Number of explants showing embryogenic calli/Total number of explants cultured) × 100.

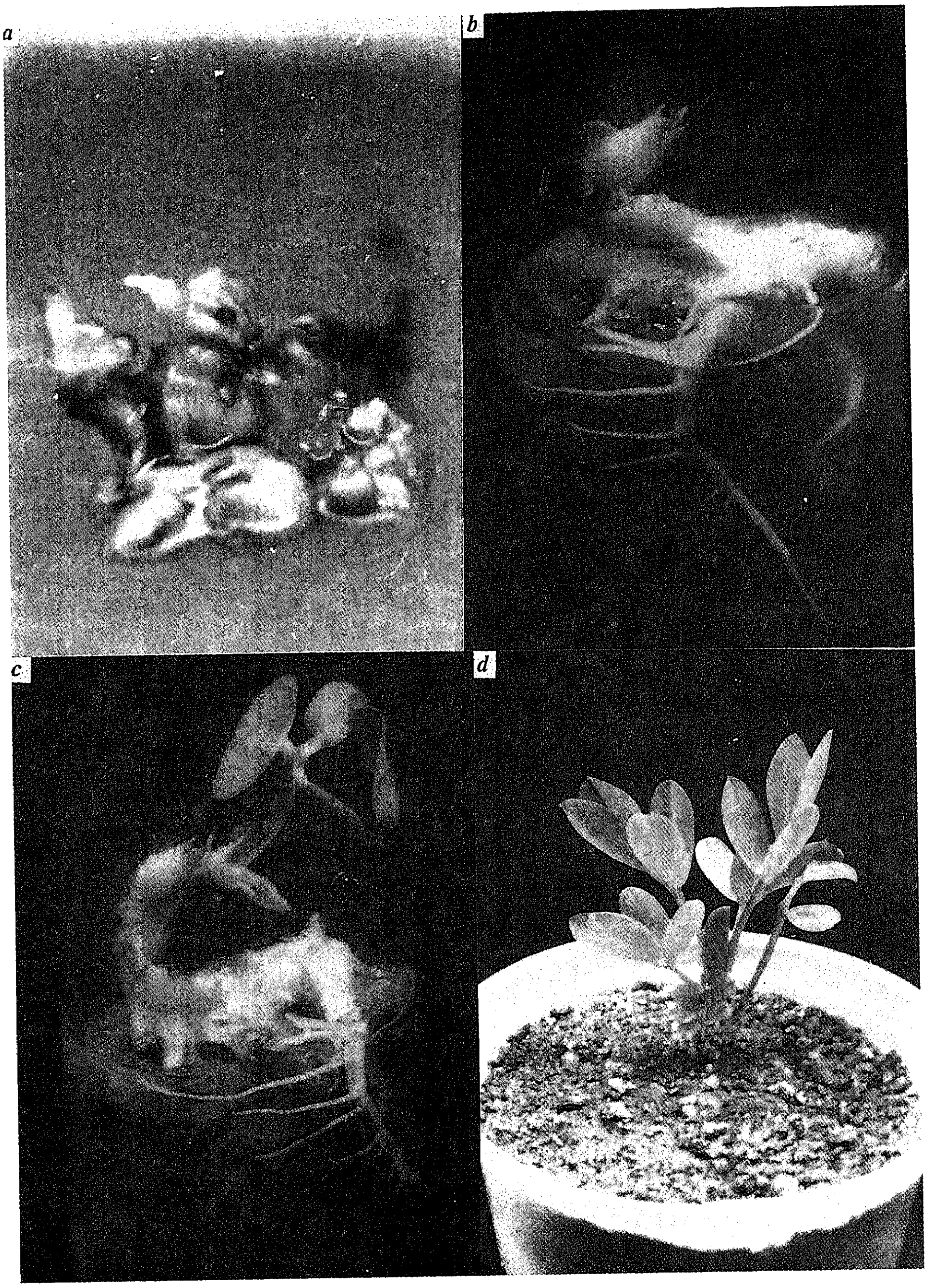

Figure 1 *a–d*. Development of plants through somatic embryogenesis in *Arachis hypogaea*. ***a***, Differentiation of embryos from hypocotyl explant on MS medium supplemented with 20 mg/l 2,4-D and 0.5 mg/l BAP; ***b***, Germination of embryos on MS medium containing BAP (2.0 mg/l) and NAA (0.25 mg/l); ***c***, Plantlet development from somatic embryos; ***d***, Regenerated plant established in plastic cup.

After 5 weeks on induction media, embryogenic mass from the preliminary experiment was transferred to the maturation medium containing 3% (W/V) sucrose, different concentrations of BAP (0.5–2.0 mg/l) and 2,4-D (0.5 mg/l) and solidified with 0.7% (W/V) agar and adjusted to pH 5.8 prior to autoclaving. The cultures were subjected to a photoperiod of 16 h light (40 $\mu Em^{-2} s^{-1}$) and incubated at 24 ± 2°C. The percentage of embryogenic calli with mature embryos was calculated as follows: (Number of embryogenic calli showing mature embryos/Total number of embryogenic calli cultured) × 100.

After reaching the dicotyledonary stage, the embryos were individually transferred to MS medium containing different concentrations of BAP (0.5–2.0 mg/l) in combination with NAA (0.25 mg/l), 2% (W/V) sucrose and 3% (W/V) activated charcoal for germination and conversion into plantlets. The cultures were incubated for two weeks at 24 ± 2°C under cool-white fluorescent light at $40\ \mu E\ m^{-2}\ s^{-1}$ with 16 h photoperiod. The conversion frequency was calculated as follows: (Number of embryos germinated/Total number of embryos cultured) × 100.

Fully germinated embryos were transferred to growth regulator free MS medium with 0.7% (W/V) agar for further elongation of shoots and roots. Cultures were incubated for 10 to 15 days in test tubes with 15 ml medium, under conditions described for germination of somatic embryos. Plants with well-developed roots were transferred to plastic cups containing a 1:1:1 mixture of sand, red soil and manure. Each plantlet was covered with a polyethylene bag and the cups were maintained in controlled temperature at 60% relative humidity. Well established plants from cups were transferred to the field. Regenerated plants were grown to maturity that produced normal flowers and set viable seeds in pods.

Statistical computations were performed using computer software. The cultures were observed periodically and the somatic embryo induction, maturation and germination percentages were recorded on the basis of visual observations. Wherever possible, the effects of different treatments were quantified on the basis of percent cultures showing the response per culture. The experimental design was random and factorial with auxin and cytokinin as independent variables. The data pertaining to percentage of embryo induction, maturation and conversion were subjected to analysis of variance (ANOVA) test. Mean separation was done using Duncan's New Multiple Range Test (DNMRT).

Highly organized, round, creamish to white coloured protuberances differentiated from all over the surface of the hypocotyls when cultured on MS medium containing different concentrations of 2,4-D or NAA (1–30 mg/l) in combination with BAP (0.5 mg/l). The epidermal cell layer of the hypocotyl split up and organized structures emerged from the subepidermal layers. The number of organized structures was higher at the cotyledonary nodal region compared to the rest of the hypocotyl. These organized structures closely resembled globular and heart-shaped somatic embryos (Figure 1 *a*). 2,4-D at a concentration of 20 mg/l produced the highest frequency of cultures showing somatic embryogenesis (62.7%) and highest average number of somatic embryos per hypocotyl explant in VRI-2 which was statistically significant at 1% level (Table 1). However, in cv TMV-7, 20 mg/l of 2,4-D induced the highest frequency of responding cultures (50.3%) and induced the highest number of somatic embryos (Table 1). When NAA was used at 1, 5, 10, 15, 20, 25 and 30 mg/l, the frequency of response varied from 9.2 to 40.5% and 7.2 to 34.2% in VRI-2 and TMV-7 cultivars respectively and average number of somatic embryos per hypocotyl ranged from 3 to 11 and 2 to 9 in VRI-2 and TMV-7 cultivars respectively (Table 1).

Table 1. Frequency of somatic embryos from hypocotyl explants of *Arachis hypogaea* cultured on a medium containing different concentrations of 2,4-D and NAA in combination with 0.5 mg/l of BAP

Hormonal concentration (mg/l)	Per cent of somatic embryogenesis		Mean no. of embryos/explant ± SD	
	VRI-2	TMV-7	VRI-2	TMV-7
2,4-D				
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5.0	10.4 ± 1.3^{f}	11.3 ± 1.4^{f}	4.0 ± 0.5^{e}	4.0 ± 0.5^{d}
10.0	24.7 ± 2.4^{e}	22.6 ± 2.1^{de}	8.0 ± 1.0^{d}	7.0 ± 0.8^{bc}
15.0	44.5 ± 3.2^{b}	41.7 ± 3.6^{b}	11.0 ± 1.2^{c}	10.0 ± 1.1^{b}
20.0	62.7 ± 5.3^{a}	50.3 ± 4.7^{a}	18.0 ± 1.9^{a}	15.0 ± 1.4^{a}
25.0	40.2 ± 3.6^{c}	37.4 ± 3.2^{c}	14.0 ± 1.2^{b}	12.0 ± 1.1^{b}
30.0	30.6 ± 2.8^{d}	26.9 ± 2.4^{cd}	12.0 ± 1.1^{c}	10.0 ± 1.2^{b}
NAA				
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
1.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
5.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
10.0	9.2 ± 1.3^{f}	7.2 ± 1.1^{f}	3.0 ± 0.6^{e}	2.0 ± 0.5^{d}
15.0	21.3 ± 2.8^{e}	18.4 ± 1.8^{de}	7.0 ± 0.9^{d}	4.0 ± 0.8^{d}
20.0	40.5 ± 3.6^{c}	34.2 ± 3.0^{c}	11.0 ± 1.4^{c}	9.0 ± 1.1^{b}
25.0	32.4 ± 2.9^{d}	29.3 ± 2.6^{c}	8.0 ± 0.6^{d}	7.0 ± 0.8^{bc}
30.0	21.4 ± 2.3^{e}	23.6 ± 2.1^{c}	8.0 ± 0.7^{d}	8.0 ± 0.9^{b}

Mean separation using Duncan's New Multiple Range Test, means within column with different letters are significant at 1% level.

For further development, globular and heart-shaped somatic embryos obtained from hypocotyls were transferred to MS medium supplemented with 3% (W/V) sucrose, different concentrations of BAP (0.5–2.0 mg/l) in combination with 0.5 mg/l 2.4-D. About 80% of the globular and heart-shaped embryos of both the cultivars developed into torpedo and cotyledonary stages in this medium. The medium without BAP did not promote the growth of the embryos. Such embryo development was observed in all combinations with varying frequencies. The highest frequency of embryo maturation (VRI-2:82.7% and TMV-7:74.3%) was observed on a medium containing 2 mg/l BAP in combination with 0.5 mg/l 2,4-D in both the cultivars, which was highly significant (Table 2). Embryos were very loosely held on the surface of calli and matured synchronously. Maturation increased with the increase in the BAP concentration. After isolation of mature embryos from both cultivars, they were transferred to MS medium containing 2% (W/V) sucrose, different concentrations of BAP (0.5–2.0 mg/l) in combination with NAA (0.25 mg/l) and 0.3% (W/V) activated charcoal for germination. Germination of cotyledonary stage embryos varied with different concentrations of BAP. Germination of embryos was characterized by simultaneous production of root and shoot systems (Figure 1 *b*). Embryos induced from hypocotyls in the presence of 2,4-D or NAA and transferred to germination medium turned green and developed by elongation of hypocotyl revealing the folded cotyledon. Later, cotyledons unfold and the shoot region developed further with the emergence of the first leaves. When MS medium was used with different concentrations of BAP (0.5–2.0 mg/l) in combination with NAA (0.25 mg/l), both shoot and root pole developed in all the combinations with varying frequencies. However, BAP (2.0 mg/l) and NAA (0.25 mg/l) combination was found to be best for highest frequency of embryo germination (VRI-2:81.7% and TMV-7:73.7%) in both the cultivars which was significant at 1% level (Table 3). Thus, BAP was necessary for germination of somatic embryos of the cultivars used in the present investigation. Germinated embryos produced good shoot and root systems when transferred to growth regulator free MS medium (Figure 1 *c*). Germinated embryos developed into complete plantlets on MS basal medium within 2 weeks. Regenerants were successfully transferred to the plastic cup (Figure 1 *d*) and subsequently transferred to the field. Properly hardened plants flowered normally and set viable seeds like that of seed grown plants (controls).

Table 2. Effect of BAP level in combination with 0.5 mg/l 2,4-D on embryo maturation from hypocotyl derived embryogenic calli of two groundnut cultivars

BAP concentration (mg/l)	No. of calli plated		No. of calli showing mature embryos		Embryo maturation response ± SD	
	VRI-2	TMV-7	VRI-2	TMV-7	VRI-2	TMV-7
0.0	32	26	0.0	0.0	0.0 ± 0.0	0.0 ± 0.0
0.5	34	29	8.6	6.1	25.3 ± 2.3^{d}	21.2 ± 2.5^{d}
1.0	38	32	17.1	13.2	45.2 ± 3.5^{c}	41.5 ± 3.8^{c}
1.5	42	35	25.7	19.3	61.2 ± 4.7^{b}	55.4 ± 5.4^{b}
2.0	45	41	37.2	30.4	82.7 ± 6.8^{a}	74.3 ± 5.4^{a}

Mean separation using Duncan's New Multiple Range Test, means within column with different letters are significant at 1% level.

Table 3. Effect of different concentrations of BAP in combination with 0.25 mg/l of NAA on germination of somatic embryos derived from hypocotyl explants into complete plantlets in two groundnut cultivars

BAP concentration (mg/l)	No. of mature embryos plated		No. of embryos germinated		Conversion per cent ± SD	
	VRI-2	TMV-7	VRI-2	TMV-7	VRI-2	TMV-7
0.0	52	54	0	0	0.0 ± 0.0	0.0 ± 0.0
0.5	58	61	13	11	22.4 ± 1.8^{d}	18.0 ± 1.3^{d}
1.0	65	68	27	25	41.5 ± 3.6^{c}	36.7 ± 3.1^{c}
1.5	74	75	46	44	62.1 ± 4.1^{b}	58.6 ± 4.4^{b}
2.0	82	80	67	59	81.7 ± 5.7^{a}	73.7 ± 4.9^{a}

Mean separation using Duncan's New Multiple Range Test, means within column with different letters are significant at 1% level.

Present studies have shown that it is possible to induce somatic embryogenesis and plant regeneration from hypocotyl explants of known commercial groundnut cultivars. Immature cotyledons[6,9], immature leaf[7,8] and immature embryos[11,16] have been successfully used for somatic embryogenesis in groundnut. Plantlet formation remained a problem in conversion of somatic embryos to plants, after transfer to basal medium, (ranged from 0 to 18%) in all the previous experiments[8–10]. In groundnut cultivars, 2,4-D was found to be the auxin of choice for the induction of somatic embryogenesis although NAA was also effective[10,17].

In media supplemented with 2,4-D (1.0–30 mg/l), clusters of somatic embryos developed from the cotyledon part of the adaxial side of the hypocotyl. This was accompanied by callusing. In media containing lower concentrations of NAA (1, 5 mg/l), callusing was observed from the cotyledon part of the hypocotyl without any sign of embryogenesis. However, at higher concentrations (10–30 mg/l), clusters of yellowish green somatic embryos differentiated from the cotyledonary nodal region of hypocotyl. 2,4-D and NAA[8,10,11,14] were widely used for somatic embryo induction in groundnut. Addition of cytokinin (BAP or KIN) to the medium promoted growth, development, maturation and conversion into plants of somatic embryos in culture. Cytokinins are known to enhance shoot morphogenesis. However, when cytokinins were used along with auxin, it showed positive effect on somatic embryogenesis in groundnut[13,16]. Embryogenesis was induced with a frequency of 40.5% with 11 somatic embryos/explant on MS medium containing 20 mg/l NAA and 0.5 mg/l BAP. However, higher concentrations (25 to 30 mg/l) of 2,4-D or NAA in combination with 0.5 mg/l BAP decreased the embryo induction frequency as well as number of somatic embryos/explant. Reddy and Reddy[16] and McKently[18] similarly observed somatic embryos on 2,4-D and BAP containing medium. The effectiveness of an auxin type appears to be tissue and species specific.

In the present study, frequency of embryogenesis and number of embryos per explant decreased with increasing concentrations of auxin level beyond 20 mg/l. McKently[18] found in embryo axis cultures, that as auxin concentrations increased (1–10 mg/l), the probability of obtaining a normal-shaped groundnut somatic embryo decreased. Higher concentrations of auxin not only decreased the embryos/explant but also delayed the embryogenesis[16]. Present studies have shown that conversion of somatic embryos into plants was dependent on the type and concentration of auxin used in the somatic embryo induction medium. The best plant conversion frequency (81.7%) was obtained when 2,4-D was used for somatic embryo induction from hypocotyl. Among the various cultivars studied in India[6,8,10,13] so far and in the present study, somatic embryogenesis seems to be better suited for genetic manipulation studies, because of high frequency of plant conversion, etc. The frequency of somatic embryogenesis in the present study was found to be the result of interaction between genotype and the type and concentration of auxin used. Parrott *et al.*[19] found that the genotype had a significant effect on the ability of immature soybean cotyledons to undergo the auxin-stimulated somatic embryogenesis.

In conclusion, an efficient protocol was developed for high frequency somatic embryogenesis using a simple medium. The present protocol has a distinct advantage over the earlier published protocols, the explant is hypocotyl by germinating seeds, which is available throughout the year.

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Influence of metalaxyl on *Glomus fasciculatum* associated with wheat (*Triticum aestivum* L.)

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Soil incorporation of metalaxyl [methyl *N*-(2-methoxyacetyl)-*N*-(2,6,xylyl)-DL-alaninate] significantly enhanced root colonization of the vesicular–arbuscular (VA) mycorrhizal fungi *Glomus fasciculatum* associated with wheat. The stimulatory response of VA mycorrhizal fungi to low concentration of metalaxyl resulted in increased plant biomass production, nutrient uptake and grain yield of wheat. However, higher concentrations of metalaxyl, particularly 2.5 ppm of metalaxyl affected the mycorrhizal infection and seed yield of wheat. Addition of urban compost to an extent ameliorated the toxic effect of fungicide on VA mycorrhizal colonization, plant growth and yield of wheat when compared to unamended soil.

THE role of agro-chemicals on vesicular–arbuscular mycorrhizal development and efficiency requires better understanding because mycorrhizal fungi are necessary components of most plant systems, and have significant effect on plant growth, physiology and nutrition. Several workers[1–3] have reported the adverse effect of systemic and nonsystemic fungicides to VA mycorrhizae. Recently, considerable attention has been given to a systemic fungicide metalaxyl [methyl *N*-(2-methoxyacetyl)-

Table 1. Effect of metalaxyl on per cent mycorrhizal colonization of wheat

Treatments	Fungicide dose (ppm)	Days after planting 40	80	120	Mean
Without compost	Control	18.00	45.00	64.00	42.33
	0.5	21.00	52.00	69.00	47.33
	1.0	26.00	38.00	76.00	46.67
	2.5	12.00	25.00	43.00	26.67
	Mean	19.25	40.00	63.00	40.75
With compost	Control	29.00	58.00	73.00	53.33
	0.5	28.00	49.00	78.00	51.67
	1.0	16.00	63.00	70.00	49.67
	2.5	22.00	31.00	42.00	31.67
	Mean	23.75	50.25	65.75	46.58

	SEm±	LSD (0.05)
Stages	2.4688	6.8431
Compost	2.0158	5.5874
Treatments	2.8507	7.9018

Table 2. Effect of metalaxyl on dry matter production (g plant^{-1}) of wheat

Treatments	Fungicide dose (ppm)	Shoot Days after planting 40	80	120	Mean	Root Days after planting 40	80	120	Mean
Without compost	Control	0.440	2.884	4.336	2.553	0.154	0.306	0.842	0.434
	0.5	0.490	3.161	5.318	2.989	0.158	0.311	0.777	0.415
	1.0	0.360	1.818	3.460	1.879	0.124	0.259	0.708	0.363
	2.5	0.383	1.282	3.512	1.725	0.128	0.241	0.656	0.341
	Mean	0.418	2.286	4.156	2.286	0.141	0.279	0.745	0.388
With compost	Control	0.548	2.642	6.081	3.090	0.177	0.355	1.587	0.706
	0.5	0.543	2.498	5.511	2.850	0.161	0.377	1.247	0.595
	1.0	0.478	2.256	6.031	2.921	0.148	0.230	1.215	0.531
	2.5	0.420	1.550	2.333	1.434	0.130	0.238	0.524	0.297
	Mean	0.497	2.236	4.989	2.573	0.154	0.300	1.143	0.532

	SEm±	LSD (0.05)	SEm±	LSD (0.05)
Stages	0.0844	0.2341	0.0087	0.0240
Compost	0.0690	0.1911	0.0071	0.0196
Treatments	0.0975	0.2703	0.0100	0.0277

N-(2,6,xylyl)-DL alaninate] and its influence on VA mycorrhizal colonization. Metalaxyl is an acylalanine fungicide that controls root rot incited by Oomycetes of the order Peronosporales. Soil incorporation of metalaxyl has been reported to increase VA mycorrhizal colonization in some of the economically important crops[4–6]. The present experiment was carried out to evaluate the effect of different concentrations of metalaxyl on VA mycorrhizal colonization, plant growth and yield of wheat.

Sandy loam soil was collected from an uncropped grassy area of the Indian Agricultural Research Institute farm, New Delhi, and passed through a 2 mm sieve. The preliminary analysis of the soil used showed 0.18% organic carbon, 0.026% total nitrogen, 3.2 kg ha^{-1} available phosphorus, 140 kg ha^{-1} available potash and the pH was 7.2. Metalaxyl was obtained from Ciba-Geigy, Switzerland. The soil was treated with 0.5 ppm, 1.0 ppm and 2.5 ppm of metalaxyl prior to sowing. Soil amendment was done with 1% urban compost to mitigate the deleterious effect of fungicide. Urban compost was obtained from the mechanical compost plant, Okhla, Delhi. The proximate analysis of urban compost is given as follows: Organic carbon 16.86%, total nitrogen 0.42%, available phosphorus 0.037%, water holding capacity 82.73% and the pH 8.10.

Seeds of wheat (*Triticum aestivum* L.) cultivar HD-2428 and culture of VA-mycorrhizae *Glomus fasciculatum* were obtained from the Cereal Laboratory and the Division of Microbiology, IARI, New Delhi, respectively. Plants were grown in earthen pots (13″ × 12″) containing 10 kg of ground and sieved soil. Undamaged, healthy and uniform seeds were surface sterilized by rinsing in 95% ethanol in a clean sterile beaker for one min, followed by immersion in 0.2% acidified $HgCl_2$ for 5 min and washed in several changes of sterile distilled water.

The VA mycorrhizal fungus was inoculated by the layering method[7]. The inoculum consisting of ground and dried sand: soil mixture containing infected root segments and chalamydospores were used at the rate of 100 g pot^{-1}. The inoculum was placed in layers or pads at a depth of 3.5 cm from the top, over which a thin layer of soil was spread. Holes were made to dibble the seeds. Before dibbling the seeds, small quantity of VA mycorrhizal inoculum was added beneath the seeds so as to enable the inoculum to come in contact with emerging roots. The plants were watered on alternate days to near-field capacity. Inoculated controls were maintained without fungicide. The plants were harvested after 40, 80 and 120 days of growth. At each harvest, per cent mycorrizal infection, dry weight of shoots and roots were recorded and the representative samples were analysed for N and P. The concentration of P was estimated by the vanadomolybdate method after digestion with tri acid mixture. Similarly, the nitrogen content was determined by the kjeldahl method[8]. After harvesting, part of the root system was cleared and stained[9] and the per cent mycorrhizal colonization was assessed and calculated from the frequency distribution method[10].

The present investigation revealed that low concentration of metalaxyl resulted in an increased mycorrhizal colonization of wheat roots at all the stages of observation (Table 1). Previous studies reported[5,11] that use of metalaxyl at recommended field rates either increased or had no adverse effect on mycorrhizal colonization associated with maize and sorghum respectively. Though the precise mechanisms by which the fungicide stimulates the VA mycorrhizal fungi are unknown[12], probably

Table 3. Effect of metalaxyl on grain yield, nitrogen and phosphorus[3] uptake (g plant^{-1}) at harvesting stage of wheat

Treatments	Fungicide dose (ppm)	Grain yield	N uptake	P uptake
Without compost	Control	8.690	0.164	0.045
	0.5	9.050	0.160	0.046
	1.0	5.280	0.087	0.022
	2.5	5.230	0.085	0.027
With compost	Control	9.560	0.201	0.055
	0.5	9.010	0.200	0.060
	1.0	8.390	0.180	0.049
	2.5	5.450	0.082	0.030
	SEm±	0.0160	0.0003	0.0002
	LSD (0.05)	0.0460	0.0010	0.0006

metalaxyl enhances the VA mycorrhizal infection by reducing the population of plant pathogens and also other soil microbes which are responsible for suppression of root colonization, thus allowing greater invasion of roots by VA mycorrhizal fungi[13,14].

The stimulatory response of VA mycorrhizal fungi to low concentration of metalaxyl resulted in the increase in biomass (Table 2), nutrient uptake and grain yield of wheat (Table 3). Similarly Afek *et al.*[15] observed an increased VA mycorrhizal colonization of roots and plant biomass in the presence of metalaxyl. The primary mechanism which is responsible for stimulation of plant growth by VA mycorrhizal fungi is due to increased uptake of phosphorus and other nutrients. The possibility of plant growth harmones being involved in such a response has also been suggested[16]. Further, Barea and Azcon-aguilar[17] observed the substances with the properties of gibberellic acid and cytokinin in the extract of *Glomus mosseae* culture.

The reduction of VA mycorrhizal infection and plant growth at higher level of metalaxyl were attributed to suppression of the mycorrhizal fungus by the fungicide. Jabaji Hare and Kendrick[12] have reported the inhibition of mycorrhizal colonization of leek roots by metalaxyl and also the fungicide significantly decreased growth of plants with time. The effect of metalaxyl on mycorrhizal infection may be a direct action or mediated through phytotoxic effect on root function and growth. In an experiment[18], application of metalaxyl at a rate of 200 μl/l reduced the plant biomass of prairie grass in steamed and nonsterile soils, the results indicated that higher level of fungicide application was phytotoxic. It was observed that the amendment of one per cent urban compost to an extent ameliorated the toxic effect of metalaxyl on VA mycorrhizal infection, plant growth, nutrient uptake and yield of wheat when compared to unamended soils. Schiavon *et al.*[19] and Stearman *et al.*[20] reported that organic matter reduces the phytotoxicity of agro-chemicals, whereas fulvic acid and humin helps in detoxification through formation of low energy bonds. Further, organic matter provides more sites for adsorption to the pesticides, thus reducing its amount in soil solution and protecting microorganisms from the toxic effects.

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QED: The Jewel of Physics. G. Venkataraman. Universities Press, 3–5–820, Hyderguda, Hyderabad 500 029. 1994. 133+viii pp. Price: Rs 65.

Eugene Wigner delivered a lecture in 1960, 'The unreasonable effectiveness of mathematics in the physical sciences'. Since then, this happy phrase has resounded through the literature of the exact sciences as the laying down by the physical scientist of the articles of his creed. For all this, mathematics has shown itself on occasion to be an unreliable ally, demonstrating a perverse willingness to comprehensively wreck the most elaborately constructed theory. And one of the most shocking instances of the breakdown of this *entente cordiale* in this century occurred during the development of quantum mechanics. To set the scene briefly, in 1933 Dirac's landmark paper had appeared, in which he initiated the second stage in the development of quantum theory by quantizing the electromagnetic field of Maxwell; and its relativistic version followed shortly thereafter. But no sooner had the new theory been erected, than it promptly loosed a 'plague of infinities' on its hapless creators. In this book under review, the second of his three-part history of quantum theory, Venkataraman takes up the story at this point and follows it to the late forties, when sanity was restored to the theory to the extent where it at least allowed a functional facility with calculations, by exorcising the infinities from it. It is also the story of three extraordinary physicists and their diverse personalities, who in large part were personally responsible for restoring quantum mechanics to its state of grace.

The book opens with a summing up of the topics treated in the first part, 'The breakthrough'. The rehash is occasionally seasoned with some of the more exotic spices of physics' larder. There is for instance, Feynman's theory of the electron moving back in time. (As in the earlier volume, Feynman's spirit animates the present one, stalking through it as a ubiquitous presence. In actuality, he may have been one of the three men who had cut quantum theory's Gordian knot. But Feynman's stature has grown through legend in popular imagination, till the exuberant vitality of his genius has been allowed to preside with the magisterial dignity of an elder statesman over the whole of quantum electrodynamics.) This introductory chapter – rather surprisingly a fifth of the book in length – begins with a discussion of the expression for the energy of an electromagnetic field. This expression is transformed into an equivalent form (by minding one's p's and q's, literally) in which it is analogous to the Hamiltonian of a one-dimensional oscillator. Apropos this procedure, it has to be remarked that here, as elsewhere, the author appears understandably defensive about importing some high voltage mathematics into the text – the inevitable occupational hazard of an undertaking like the present one, which aims at a level roughly between a popularization and a textbook. Thus, with a quick apologetical murmur about 'a bit of algebra', the end result is produced (page 4) with the air of a conjuror materializing a rabbit out of a top hat. (One is irresistibly reminded of the eponymous hero of Salman Rushdie's *Haroun and the Sea of Stories*, who, when asked for a clarification, disarmingly confesses it to be a P2C2E – a process too complicated to explain!)

A similar observation could perhaps be made about a cascade of undefined terms – e.g. relativistic covariance, energy of a radiation field, coupling energy, zero point energy, etc. – in the opening chapter itself. While on the subject, it has to be pointed out that if there is an Occam's Razor for writers, it would surely admonish aspiring authors not to proliferate jargon beyond strict necessity. For instance, it does not add anything to a reader's enlightenment that the symbol A (in page 7) is called the vector potential of a magnetic field. To paraphrase the little girl who was immortalized for her adventures in Wonderland, that is rather a lot for a symbol to mean.

The second chapter introduces the Feynman diagrams – the technique that Feynman invented to understand the mechanism of particle interactions. These schema constitute Feynman's abiding contribution to the language of modern theoretical physics. A large part of this chapter is devoted to developing familiarity with manipulating these diagrams and extracting information from them. The ideas are set out with all the beautiful clarity, which is the hallmark of Venkataraman's writing. This is in spite of a few minor lapses – the diagram 2.2 for instance, has been wrongly labelled. Finally, near the end of the chapter, we are shown the mathematical sleight-of-hand by which Schwinger, Feynman and Tomonaga tamed the infinities that the Dirac equation had unleashed.

The following short chapter presents the three pieces of evidence which clinched the issue in favour of QED and launched it into its career as the most successful scientific theory of all times – the Lamb's shift in hydrogen, the anomalous magnetic moment of the electron and the splitting of the ground state of positronium. But what features of the new theory ensured this resounding success? In the fourth and longest chapter of the book, Venkataraman takes up this question and shows that strict conformity at every stage with the requirements of relativity was the crucial ingredient of the new recipe. In Oppenheimer's perception, 'not merely the field equations themselves, but the whole method of approximation and solution must at all stages preserve relativistic covariance'. A review of the theory of path integrals and renormalizability concludes the chapter. One reflection on the use of equations in this book – Stephen Hawking's publisher reportedly advised him that with every equation he introduced into his book, he was halving his potential audience. One rather sees his point when one comes across instances of equations like,

$$\psi(t) = \psi(t_0) - (i/h)\int_{t_0}^{t} \psi(t')\, H_I(t')\mathrm{d}t',$$

(in page 69) which are mere shotgun marriages of the left and right hand sides performed by the equality symbol as the officiating priest. There are perhaps inevitable constraints when advanced scientific theories are discussed at popular levels of exposition. Unfortunately, the arduousness of such an undertaking cannot absolve Venkataraman from the more serious lapse of using quotations without giving references – rather disconcertingly like plucking apples from an invisible tree.

At this stage, much like an alpine guide pausing to point out features of local interest to a tourist group while waiting for the stragglers to catch up (and the reviewer must confess himself to be of this company), the author breaks the narrative with a biographical interlude of the three architects of QED. Incidentally, for anyone interested in the Feynman story, James Gleick's *Genius* must be mandatory reading – as much for the portrait of the whimsical genius of one of the most extraordinary scientists of all times as for the wealth of its anecdotal detail. In the final quarter of the book, some historical and biographical footnotes bring to a close this excellent account. It is a presentation written with a vigour and verve which do justice to one of the most turbulent epochs in theoretical physics. And, in this as in his other books, Venkataraman has established and maintained a high level of professional rigour in a field which has remained largely neglected in this country.

And what are we to say of QED? Richard Feynman was as clear in his assessment of it as he was in its affirmation, when he called it 'the jewel of physics'. But philosophically, he was an operationalist to whom the quintessence of science resided in its predictive capability. And if he could find an equation which could do just that, he would not concern himself with ontological enquiries into the nature of physical reality – an attitude entirely consonant with an age which had derived its philosophical orientation from logical positivism and the Copenhagen Interpretation. Dirac, on the other hand, died believing that the problems raised by self energy had merely been papered over by a theory cobbled together to permit computational facility. In the final analysis, it must be clear that the last word has not been said about the quantum. A cheerful acceptance of inevitable ignorance might perhaps not be a bad attitude with which to ride out this age of uncertainty – rather like old Kaspar in Southey's *Battle of Blenheim*.

> 'But what good came of it at last'
> Quoth little Peterkin.
> 'Why, that I cannot tell', said he,
> 'But, 'twas a famous victory'.

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Banking on Biodiversity: Report on the Regional Consultation on Biodiversity Assessment in the Hindu Kush–Himalayas. Pei Shengji, ed. International Centre for Integrated Mountain Development, Kathmandu, Nepal. Price not known.

Managing biodiversity is managing contradictions. This is more so in frontier areas where international issues become additional problems. At the same time, knowing the fact that the more mobile components of biodiversity do not respect political boundaries, it is necessary for the nations to cooperate if at all they want to see success in the conservation of biodiversity especially in the border areas. Viewed from this perspective, this book is a timely contribution to society.

This book is the outcome of a workshop, on the assessment, monitoring, and management of the biodiversity of the Hindu Kush–Himalayas (HKH) region, that was organized by International Centre for Integrated Mountain Development (ICIMOD) in December 1995 at Kathmandu, Nepal. Experts from eight HKH countries, namely, Afghanistan, Bangladesh, Bhutan, China, India, Myanmar, Nepal, and Pakistan participated. The participants reviewed the status of biodiversity in the region and made recommendations about future plans of actions for each country.

The book is organized into three parts. The first part spells out the proceedings of the workshop and working groups' reports on the issues and needs of biodiversity management in the HKH region, the second contains the country review papers from experts of eight HKH countries and the third contains seminar papers from experts representing international organizations engaged in biodiversity conservation. The book also contains 26 beautiful colour photographs of wildlife.

The title of the book gives a mistaken impression that it contains information about the biodiversity of those HKH regions on which people rely for their future, while the contributors, in their respective country reports, talk about more than what is present in HKH regions of their respective countries. This extra information is welcome in the sense that it informs us about what we have and what we do not. The country reports give an overall review of the current state of biodiversity in the respective countries.

The HKH region is ecologically highly diverse as it covers polar-like environments and cold deserts to the rainforests. Mountain regions are rich in biodiversity because of their inherent environmental heterogeneity. At the same time, they are fragile and prone to degradation due to disturbances. Most of the country reports emphasize this fact. However, a limited human intervention enriches the biodiversity of the mountain regions also.

This book will be useful for biogeographers, policy makers, environmentalists, nature enthusiasts, soldiers of the green brigade, teachers and environmental awareness generators. Initially it looks less useful for the technical professionals who like to go deep into the functioning and processes of nature and then devise measures for the better functioning of the system. However, any technical advice will have to be implemented taking into consideration the other ground realities of the system. A number of such points and issues have been discussed well in this book. Experiences based on the effect of Afghan war on biodiversity and its conservation, importance of predators in rodent (pest) control in Afghanistan, effect of deteriorating law and order and erosion in the sense of value in Bangladesh, emphasis on maintenance of essential ecological processes, and the work of international organizations like WWF, IUCN, IBPGR (now IPGRI) in various countries in conserving biodiversity, could prove useful for technical professionals.

The quality of write-up, information given and the presentation from various contributors to the book are indicators of the technical know-how, scientific capabilities and the work already done in various HKH countries. China and India have done relatively well in this regard and have vision and future plans like undertaking integrated regionalization of biota, replanning of nature reserves on the basis of the regionalization and overall regional considerations. Small countries also have success stories and experiences of all sorts that will be very useful to the policy makers and the researchers. Most of the countries have emphasized studies on species of economic importance and their wild relatives. Simultaneously, the HKH region has a number of botanical curiosities to offer to us.

In most of the HKH countries the earlier plant explorers were the westerners and at present also there is shortage of taxonomic expertise in these countries mainly because of lack of funds, motivation and mismanagement. Examples of the experiences of small countries include the success of the great one-horned rhinoceros in Royal Chitwan National Park in Nepal, and wildlife conservation in the Bar Valley with the help of locals in Pakistan. However, the author (contributor) from Pakistan seemed to be better experienced about the factors causing loss of biodiversity. He was even suspicious about the process of economic development itself which, he thinks, could widen the inequality and force the poor to depend more on natural resources. Pakistan's experience, of the effects of the Afghan war on its biodiversity in the regions where Afghan refugees came, the effect of the hunting culture of Mughal emperors and the hangover of this culture (a number of its wild animals hunted to local extinction), could be seen in the book. A number of other factors like low literacy, absence of respect for law, lack of political commitment and environmental awareness, etc. have added to such local extinctions. Such local extinctions would add to global extinctions about which all conservationists should worry.

The seminar paper by Busby and Gordon on biodiversity information management and their call for information cooperative not only makes very good reading but also seems to be a very good idea. However, only time will tell whether such enlightened people really want to conserve biodiversity with the intention of empowering the custodians of biodiversity or want to seek monopoly on whatever is left with others. Conservation issues are quite complicated and depend on many factors. That is why there are situations when legislations convert forest use from necessity to crime. The book brings out such inherent contradictions in conservation issues. The editor and the contributors deserve hearty congratulation for their effort in educating us.

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IUCAA revisited

In September 1990, I had visited the Inter-University Centre for Astronomy and Astrophysics (IUCAA) when it was in the process of being set up as a resource centre for Astronomy and Astrophysics (A&A) in the university sector. The idea of setting up such a centre in the pleasant surroundings of the campus of the University of Pune was inspired by another major project in astronomy also being based there: the Giant Metrewave Radio Telescope of the Tata Institute of Fundamental Research.

Indeed, during this first visit, the wing of the TIFR managing the GMRT project (now named the National Centre for Radio Astrophysics) already had its buildings and, being next door to the up and coming IUCAA, had lent the latter its facilities. Thus the seminar which I went to attend was in fact held in the lecture hall of the NCRA. I recall being taken round the IUCAA site by its Director, Jayant Vishnu Narlikar who not only briefed me on the mandate of the budding inter-university centre, but also on how the buildings were being planned to achieve its aims and objectives.

To me it seemed a far extrapolation from the few activities which I saw, managed by a skeleton staff, to the full fledged institutional programme as envisaged in the ambitious project report written in 1988, the year the centre was founded. It was yet another extrapolation, from the chaos and bustle of the building construction then going on to the architectural masterpiece which was envisioned. I recall leaving for Bangalore with good wishes for this new experiment in the university sector, but also apprehensions as to its success.

A few months ago I had a chance of a second visit, now after a span of some six years. How do the vision and dreams compare with achievement and reality?

Campus

No visitor to this beautiful campus can deny that it is indeed a unique piece of architecture, where the imaginative use of spaces to promote mixing and interactions between the scientist users goes hand in hand with well-planned interiors, soothing landscaping and last but not the least, careful attention towards maintenance. A marked contrast to so many of our public institutions which are inaugurated with great fanfare but begin to decay from day one for lack of attention to such details. The director gave full credit to the architect and the various consultants and to his own colleagues for all this.

The IUCAA buildings are spread over three plots divided by two roads named after Meghnad Saha and Vainu Bappu, two Indian stalwarts in A&A. Akashganga has the staff colony and the recreation centre, Devayani houses the main office buildings and facilities of the Centre while Aditi has the Chandrasekhar auditorium and of course, the shed Aditi where the IUCAA had started its activities in 1989. The different blocks in Devayani are named after Indian astronomers of an earlier age, Aryabhata, Varahamihira, Brahmagupta and Bhaskara (see *Curr. Sci.*, 1990, **59**, 962–964).

While going round these blocks, I came across one named 'Ballava'. Who was Ballava, I asked. Jayant explained that it was the name assumed by Bhima when he went incognito in the court of Virata in the Mahabharata saga, when he played the part of the chef in the royal house. Aptly the name is given to the IUCAA canteen block! Inside the canteen one sees a sobering quotation which would bring any astronomy researcher back to earth: The discovery of a new dish does more for human happiness than the discovery of a star. Predictably, the quotation is from Brilliant-Savarin, a great chef.

Exhibits

A speciality of the campus is the integration of the architecture with scientific exhibits. Thus the centrepiece of Devayani is the Kund which has four statues, of Aryabhata, Galileo, Newton and Einstein. Behind Aryabhata, appropriately, is the entrance to the Aryabhata block with the centrepiece of the Foucault Pendulum. In his shloka in *Aryabhatiya*, Aryabhata had stated that the Earth spins about an axis and hence the fixed stars appear to move in the westerly direction. The Foucault pendulum reminds astronomers that the Earth's spin relative to an inertial frame can be measured without recourse to the stars. Newton's statue has an apple sapling specially gifted by the Brogdale Horticultural Trust: it is a plant whose ancestry can be traced to the orchard in Newton's home, the Woolsthorpe Manor.

At the centre of the Kund itself is a representation of what radio astronomers would identify as a radio source with accretion disc and jets coming out of the central compact region.

The guest house cum hostel is called Nalanda and its courtyard has the Sierpinski gasket landscaped. . . a fractal figure of Hausdorff dimension 1.58 approximately. The smallest of the three principal courtyards has two banyan trees which symbolize a binary star system whose Roche lobes and Lagrangian points are well identified.

Across the Meghnad Saha road, within the foyer of the auditorium is the aperiodic tiling whose mathematical features were highlighted in Roger Penrose's book *The Emperor's New Mind*. Under construction outside is a replica of the Hampton Court Maze whose walls are made of hedges. It is an excellent practical exercise in the connectivity of figures. Indeed there are plans to develop the outside areas into a science park. A replica of the Samrat Yantra already dominates this area.

The astronomical pictures gifted by David Malin adorn the walls on the first floor of Aryabhata while Ashwin Mehta's photographs grace the ground floor. There are the occasional wall paintings like the Lorentz attractor and Glashow's snake to which more may be added in due course.

Facilities

The IUCAA was designed to provide facilities to university academics which will help raise their productivity in A&A both in quality and quantity and provide them with new avenues of research. The excellent library, computer centre with worldwide access on the networks, the data centre which can tap on-line databases, the instrumentation laboratory which provides opportunities and guidance in do-it-yourself instrumentation in the astronomical field are housed in the north-east corner of the Devayani compound, around the smallest of the three courtyards.

The visitors need housing which is provided in the Nalanda Guest House and the Takshashila apartments. University faculty and students, participants of workshops and training programmes as well as experts from outside are housed here. With a commuting distance of less than a hundred metres to work, and the fact that academic work can go on in principle at any time of the day or night, the visitors should have no cause for complaint!

Programmes

In the project report, the IUCAA had projected its activities along an 'eight-fold way', involving simultaneous attacks on several fronts. Even when I had first visited, these activities were initiated despite small and inadequate infrastructure. IUCAA's academic calendar is published around June every year and it makes impressive reading. Apart from its own inhouse graduate school, the IUCAA faculty are guest lecturers in the astrophysics M Sc stream of the Pune University Physics Department. There are a number of schools and workshops throughout the year, ranging from the introductory to the frontier level both at the IUCAA campus and in different universities.

Thanks to its guest observing programme, IUCAA has been able to involve university academics in optical astronomy in India and abroad. Encouraged by the response and anticipating growth in the coming years, a 2-metre class telescope has been ordered with the Royal Greenwich Observatory, UK and it should be operational well before the end of this century.

The IUCAA has been getting more and more university departments interested in including A&A as a special subject in the Physics or Mathematics M Sc programmes. Thus a model syllabus has been framed in a modular form which can be adapted to the system prevailing in any particular university.

The culture of instrumentation has been catching up, thanks to the projects initiated by the instrumentation laboratory. I saw a prototype 14-inch automated photoelectric telescope being fabricated. It is being duplicated by a team from Bangalore University. Hopefully, other universities will follow this example.

There are other smaller projects relating to detectors. There are more ambitious proposals for sophisticated instruments to go with the IUCAA telescope. It is hoped that the universities will be involved along with IUCAA in these.

As a part of efforts to mobilize human resources in A&A, science popularization forms an important programme of IUCAA. In 1991, IUCAA convened a national meet of amateur astronomers. This proved to be a catalytic activity which set the pattern for future such meets convened annually in different parts of the country. Now the amateurs have formed a national confederation of different clubs. In addition, workshops for making telescopes and sky globes by IUCAA have proved popular.

An important feature of all such programmes is IUCAA's involvement with school children through lectures and summer projects. The lecture demonstrations in English, Marathi and Hindi in the IUCAA's Chandrasekhar auditorium on the second Saturday of the month are oversubscribed. In the summer vacations, over 100 children from Pune schools spend a week each at IUCAA doing some astronomy-related project with a staff member. From research students to the director, all members participate in this programme actively.

Achievements

Thus great care has gone into the planning and management of this centre and its academic programmes. This brings me to the basic question: Has the idea of such a centre worked? Are the aims and objectives of the centre given in the project report being fulfilled? There is no doubt that the work done at IUCAA is already beginning to be known internationally. The response to IUCAA's post-doctoral programme, the participation of its academics in its international programmes and conferences, and the flow of reputed scientists to the centre's academic activities have all been very encouraging.

But how about its impact on the university scene? Here the achievements have been admittedly mixed. The general feeling was that the associateship programme has helped uplift the work of some twenty per cent of the participants, while for another twenty per cent the improvement may show in another three to four years. Why are these percentages low? The reasons are many.

The university system has its own inertia. Despite exhortations to the vice-chancellors by the successive Chairpersons of the University Grants Commission to look upon the Inter-University Centres as their own field stations, very few have been liberal enough to grant suitable leave facilities to the associates to enable them to come and visit IUCAA for their work. The numerous workshops and schools do help introduce new research topics to academics from the universities, but only a percentage of the above order have taken advantage of them. Perhaps IUCAA needs to adopt a more agressive and persuasive technique to bring about changes of attitudes.

The new project of the IUCAA telescope has great potential for encouraging the observational programmes amongst universities. There will be practical difficulties like the observer getting leave at the time when his or her programme is scheduled, the paucity of student-power in the universities, etc. These are challenges to be met.

Even before the completion of the GMRT project, a community of university users in radio astronomy has to be built up. Perhaps it is time for IUCAA and the NCRA to get together to formulate a strategy that will gradually induct the universities into using the GMRT.

What of the future?

Having visited the Inter Universtiy Consortium for DAE facilities (IUC-DAEF) some years ago (*Curr. Sci.*, 1995, **68**, 670–671), one could not help asking the same questions which came up in the context of that institution. A balance has to be struck between the service activities which form the primary mandate of the institution, and the quality of research by its own scientists. If the latter suffers, then it does affect the wider goal of quality work in

A&A at the universities. My own impression, which seems to agree with that of IUCAA's Director, is that the interaction with universities has been quantitatively as planned but its quality needs to be looked into – easier said than done. Looking at the impressive calendar, one does wonder whether there is a risk of spreading oneself too thin. In the early years, the activities were somewhat top-heavy (i.e. relativity and theoretical cosmology were over-represented), and even after later expansion into other areas, one wonders whether the observational, phenomenological and instrumentational sides of astronomy are fully taken care of. Of course, one could consciously stay away from them, since there are other astronomical institutions in the country, and in fact that would be better than some sub-critical activity for the sake of maintaining a presence.

Having congratulated IUCAA for its rapid progress in the last seven years, I may be forgiven some cautionary remarks, based on my experience with at least four institutions. The initial years are in some sense a honeymoon period, when novelty and excitement act to mask the precursors of the troubles ahead. A wise institution (or couple!) would do well to anticipate some of these. Scientists are by their very nature individualists and hard to weld into a team. In fact, the problem of getting faculty to pull together and respect and take part in collective decision making is widespread. The alternative, of relying on the energy, initiative, and judgment of a single personality, does not work forever! Given the overall goal of helping the university sector, some guidelines have to be laid down and followed, so that the burden of this task does not fall on too few shoulders. One must say that by and large, the structure and functioning of IUCAA have a degree of transparency which is rare elsewhere in the country.

Ultimately, the relation of IUCAA and the Universities has to evolve to become an equal one, based on mutual respect, rather than mutual need. One knows that the world's best radio telescopes, run by the National Radio Astronomy Observatory (NRAO) in the USA, come under an organization called Association of Universities for Research in Astronomy (AURA) which is no mere figurehead, but takes a lively interest and role in the functioning of the NRAO. The dilemma of both IUCAA and IUC-DAEF is that at the moment, such inspired guidance from the universities will not be forthcoming and they will have to play the role of a guide to their own future masters. Lest such terminology seem repugnant, let us remember that Jawaharlal Nehru described himself as 'the first servant of the Indian people'. Everyone will wish IUCAA success in the difficult role that it has taken up, of being the first servant of Astronomy and Astrophysics in the Indian Universities.

S. Ramaseshan

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MINISTRY OF SCIENCE AND TECHNOLOGY
DEPARTMENT OF SCIENCE AND TECHNOLOGY

JSPS POSTDOCTORAL FELLOWSHIPS: 1997–98

The dates for requesting application forms and for their submission have been revised as follows:

Last date for receiving requests: 15 March 1997
Last date for receiving completed application forms: 15 April 1997

MEETINGS/SYMPOSIA/SEMINARS

National Workshop on Technology Management

Date: 5–6 March 1997
Place: Pune

Objectives: To encourage industry–institute partnership programme, Integration of business, technological and strategic management, Study and evaluate the temper and tempo of corporate groups after post-liberalization phase, Evolve industry–institute consensus on various emerging issues, Identification of thrust areas for joint programmes of research and development between industries and institutes.

Contact: Director
Bharati Vidyapeeth's Institute of Management and Enterpreneurship Development
Erandwane, Pune 411 038
Phone/Fax (0212) 331060

Training Programmes in Food Processing

Place: Mysore

The Central Food Technological Research Institute, Mysore is conducting residential courses on 1. Analysis of pesticide residues in foods (10.3.97–14.3.97), 2. Strain improvement of industrial microorganism mutation, protoplast fusion and recombinant DNA (24.3.97–4.4.97), 3. Oilseed processing with an emphasis on indigenous technology (7.4.97–11.4.97) and 4. Flexible packaging of fresh and processed foods (12.5.97–16.5.97).

Contact: Head, Human Resource Development Dept.
Central Food Technological Research Institute
Mysore 570 013.

Workshop on Swift Heavy Ions in Materials Science (SHIMS)

Date: 10–11 March 1997
Place: Bangalore

Topics include: High energy ions in semiconductor devices, Columnar defects in high T_c superconductors and other materials, Thin films, surfaces and interfaces – role of swift heavy ions, HIBS, ERDA, NRA – Nuclear techniques with SHI, Biomaterials, Materials modification with SHI: Polymers, Quasi-crystals, etc.

Contact: Dr D. K. Avasthi
Nuclear Science Centre, New Delhi
Fax: 011–689–3666
E-mail: info@nsc.ernet.in.

National Symposium on Herbal Medicine: Application of Biotechnology and Futuristic Approach

Date: 27–29 March 1997
Place: Dehradun

Objectives: to redefine the term herbal medicine, to create further awareness about herbal medication, to exploit herbal knowledge at industrial scale, to improve and standardize active constituents by use of biotechnology, to expedite research for designing of new medicines.

Contact: Dr Anil Kumar Puniya
Convenor, Department of Microbiology
SBS Postgraduate Institute of Bio-Medical Sciences
Balawala, Dehradun 248 161
Phone: 0135–686246

Workshop on Pesticide Toxicity Methods

Date: 31 March–11 April 1997
Place: Padappai

A two-week intensive course is designed to impart practical approaches for evaluation of pesticides for various toxicity parameters and *in vitro*, *in vivo* and molecular toxicity methods.

Contact: Director
Fredrick Institute of Plant Protection & Toxicology
Padappai 601 301
Tamil Nadu
Phone: 04111–44246, 44266
Fax: 044–2367832,2368024

National Symposium on Oceanography and Coastal Zone Management

Date: 23–24 April 1997
Place: Visakhapatnam

Topics include: Physical, chemical and biological oceanography and marine meteorology; Coastal and nearshore processes; Marine geology and geophysics; Marine pollution and monitoring; Estuarine dynamics.

Contact: Dr K. S. R. Murthy
National Institute of Oceanography
176, Lawsons Bay Colony
Visakhapatnam 530 017
Phone: 0891–554180
Fax: 0891–543595
Grams: OSTECHLAB, VISAKHAPATNAM

Institution of Chemists (India): Associateship Examination, 1998

The 48th Associateship Examination of the Institution of Chemists (India) will be held in November 1998. Further enquiries regarding this and for Membership may be made to the:

Honorary Secretary
Institution of Chemists (India)
11/4, Dr Biresh Guha Road
Calcutta 700 017

International Conference on Frontiers in Biotechnology

Date: 26–29 November 1997
Place: Trivandrum, India

Topics include: Industrial Biotechnology, Food and Pharmaceuticals and Environmental Biotechnolgy.

Contact: Dr Ashok Pandey
Biotechnology Unit
Regional Research Laboratory
Trivandrum 695 019
Phone: 91–471–490674
Fax: 91–471–490186, 491712
E-mail: pandey@csrrltrd.ren.nic.in.

The Seventh Asian Congress of Fluid Mechanics

Date: 8–12 December 1997
Place: Chennai

Topics include: Turbulence and flow instabilities, computational fluid dynamics, Gas dynamics, Boundary layer flows, Aerospace fluid dynamics, Geophysical fluid dynamics, Environmental fluid mechanics, Combustion and reacting flows, Hydrodynamics and hydraulics, Industrial fluid mechanics, Fluid machinery, Bluff-body aerodynamics, Multi-phase flows, Bio-fluid mechanics, Flow visualization, Flow measurement, Flow control, Heat and mass transfer, Astrophysical fluid dynamics, Plasma dynamics, Magnetohydrodynamics, Aeroacoustics and aeroelasticity.

Contact: Dr E. G. Tulapurkara
Department of Aerospace Engineering
Indian Institute of Technology
Chennai 600 036
Phone: 91–044–2351365 Extn. 3001/3006
Telex: 91- 044–8926 IITM–IN
Fax: 91–044–2350509/2352545
E-mail: egt@aero.iitm.ernet.in.

International Symposium on Mathematical Physics in Memory of S. Chandrasekhar

Date: December 1997
Place: Calcutta, India

Topics include: Astrophysics: Stellar structure, stellar dynamics, radio astronomy, infrared astronomy, star formation, end phase of stellar evolution; Early universe: Nucleo synthesis, inflationary theory, quantum gravity, dark matter; High energy astrophysics: White dwarfs, neutron dwarfs, supernovae, radio galaxies, active galactic nuclei, quasars and pulsars, black holes; Theory of relativity and relativistic astrophysics: Exact solution, unified field theory, Kaluza–Kline theory, cosmological models, other alternative theory; String theory: Superstring, supersymmetry; Fluid mechanics: Stability and transition, turbulence, transonic flow, boundary layer and shock wave, water waves, MHD and physico-chemical flow, geophysical and astrophysical fluid mechanics; Philosophy of mathematical physics: The problem of appraising scientific theories, anomalies versus crucial experiments, quantum logic, others.

Contact: Jt. Directors
S. N. Bose School for Mathematics and
Mathematical Science
AE–374, Sector–1, Salt Lake City
Calcutta 700 064

International Conference on Triple Helix of University–Industry–Government relations

Date: 7–10 January 1998
Place: New York City and Purchase, New York

Paper abstracts are invited. Details can be obtained from the addresses given below.

Dr Henry Etzkowitz
Science Policy Institute, Social Science Division
State University of New York at Purchase, 735
Anderson Hill Road, Purchase, NY 10577–1400,
USA
Telephone: (+1) 914 251 66001
Fax: 914–251–6603
E-mail: spi@interport.net.

Dr Loet Leydesdorff
Department of Science & Technology Dynamics
Nieuwe Achtergracht 166, 1018 WV Amsterdam,
The Netherlands
Telephone: (+31) 20–525 6598
Fax: 525 6579
E-mail: 1.leydesdorff@mail.uva.nl.

Publications received

Books received by the journal in 1996 but not reviewed are listed. The list is intended to serve as both a notice of the books and an acknowledgement of receipt. Some reviews may appear later.

Advancements in Micronutrient Research: A. Hemantaranjan (ed.). Scientific Publishers, 5A, New Pali Road, P.O. Box 91, Jodhpur 342 001. 1995. 465 pages. Rs 850.

Agricultural Inputs and Environment: S. P. Palaniappan (ed.). Scientific Publishers, 5A, New Pali Road, P.O. Box 91, Jodhpur 342 001. 1995. 445 pages, price not known.

Applied Chemistry Theory and Practice: O. P. Virmani and A. K. Narula. New Age International Private Limited, 4835/24, Ansari Road, Daryaganj, New Delhi 110 016. 1995, 321 pages. Rs 120.

Biological Diversity and Environment: M. Shamim Jairajpuri. CBS Publishers & Distributors, 4596/1A, 11 Daryaganj, New Delhi 110 002. 167 pages. US $ 25. Rs 495.

Blood Substitutes: New Challenges: R. M. Winslow *et al.* (eds). Birkhauser Verlag AG, Klosterberg 23, PO Box 133, CH-4010, Basel, Switzerland. 1996. 208 pages. DM 118.

Challenging Boundaries, Global Flows, Territorial Identities: Vol.2: M. J. Shapiro and H. R. Alker (eds). University of Minnesota Press, P.O. Box 1450, Minneapolis, AN 55485-8342, USA. 1995. 493 pages. US $ 21.95.

Clifford (Geometric) Algebras: William E. Baylis (ed.). Birkhauser Verlag AG, Postfach 133, CH 4010 Basel, Switzerland. 1996. 517 pages. DM 118.

Contributions in Environmental Geology Spl. Publn No.43: Geological Survey of India, Sector E, Aliganj, Lucknow 226 024. 1996. 261 pages. US $ 54.36/Rs 151.

Contributions to XV Indian Colloquium on Micropaleontology and Stratigraphy: Jagdish Pandey *et al.* (eds). KDM Institute of Petroleum Exploration, Dehra Dun 248 195. 1996. 827 pages. Price not known.

Control of Electrical Machines: S. K. Bhattacharya and Brijinder Singh. New Age International Private Limited, 4835/24, Ansari Road, Daryaganj, New Delhi 110 002. 1996. 356 pages. Rs 135.

Cretaceous Stratigraphy and Palaeoenvironments: Ashok Sahni. Geological Society of India, P.B. No.1922, Bangalore 560 019. 1996. 433 pages. US $ 50/Rs 500.

Decorative and Dimensional Stones of India: D. Venkat Reddy. CBS Publishers & Distributors, 4596/1A, Daryaganj, New Delhi 110 002. 1996. 276 pages. Rs 295.

Elements of Ordinary Differential Equations and Special Functions: A. Chakrabarti. New Age International Private Limited, 4835/24, Ansari Road, Daryaganj, New Delhi 110 002. 1996. 158 pages. Rs 75.

Fundamentals of Environmental Sciences: G. S. Dhaliwal *et al.* Kalyani Publishers, 1/1, Rajinder Nagar, Ludhiana 141 008. 1996. 374 pages. Rs 150.

Fundamentals of Optical, Spectroscopic and X-ray Minerology: Sachinath Mitra. New Age International Private Limited, 4835/24, Ansari Road, Daryaganj, New Delhi 110 002. 1996. 336 pages. Rs 150.

Indicators of Indian Science & Technology: Ashok Jain *et al.* Segment Books, B-23/25, Kailash Colony, New Delhi 110 048. 1996. 151 pages. Rs 695.

Inferential Problems in Survey Sampling: Parimal Mukhopadhyay. New Age International Private Limited, 4835/24, Ansari Road, Daryaganj, New Delhi 110 002. 1996. 242 pages. Rs 450.

In Search of the Cradle Civilization: George Feyerstein *et al.* The Theosophical Publishing House, P.O. Box 270, Wheaton IL 60189–0270, USA. 1995. 341 pages. US $ 24.95.

Killari Earthquake, 30 September 1993: P. K. Narula *et al.* (eds). Geological Survey of India, Sector E, Aliganj, Lucknow 226 024. 1996. 282 pages. US $ 95.40/Rs 265.

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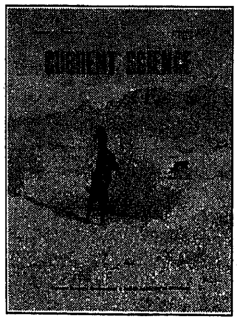

COVER. A goat, stretching its front legs up to feed on the foliage of the Kumta tree. After one or two rains, the soil in a desert turns green. (Photo: Rajesh Tandon). See page 306.

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In this issue

Life in extreme environments

Ever since the evolution of early plants about 100 million years ago (during Cretaceous), angiosperms have been invading all the habitats of the planet. They are found almost everywhere including in the Arctic, plains, deserts, swamps, freshwater rapids, salt marshes. However, not all these places can be considered habitable. Many of the environs (such as hot springs, salt marshes and deserts) constitute conditions that at best can only stifle life. But even under these conditions, plants seem to have done extremely well in evolving features of infinite complexity to adopt to the rigors of the environment, making their stay a comfortable one.

Each of the adaptations to the extremes of the environment is a story in a scientific precision and is best exemplified by plants growing in the arid deserts. For example, on a warm summer day, it is estimated that a date palm loses nearly 4000 l of water, a ragweed about 8 l and the common cactus just a couple of spoons of water. Yet all the three systems share the same basic mechanism of uptake of water. The saguaro, one of the remarkable cacti of the Sonoran desert, is believed to store about a ton of water in its stem. A single rye plant, in an attempt to locate water, puts out roots where total length in four months equals the distance between Bangalore and Madras (a promise for cellular phone facility?).

Plants growing in water (e.g. deep-water rice) are known to employ an ingenious technique for transporting oxygen from their leaves to their roots. Many species of plants nonchalantly take to salt-rich diet and thrive exceedingly well in brackish waters and salt marshes. They have elaborate machanisms to 'excrete' the excess salt through their foliage and also compartmentalize salts in their cell vacuole. Plants growing in acid soils readily extrude out calcium salts as a mechanism to neutralize the pH. The list of such adaptations seems to be unending as newer research comes to light. Indeed many of the adversities in the habitat of plants have provided a fair share of excitement to scientists to unravel the underlying mechanisms contributing to the adaptations.

In their article on **page 306**, Mohan Ram and Gupta provide a fascinating account of the natural history of plant life under extreme environment and provoke frequently the necessity to understand the mechanisms contributing to their adaptations.

R. Uma Shaanker

Thyroid hormones

Thyroid hormones and their actions have commanded a separate identity in endocrine literature particularly from a comparative endocrinology perspective. Along with glucocorticoides and pituitary prolactin they are considered 'permissive hormones'. A molecular correlate of 'permissiveness' has eluded researchers in this area. A uniform biochemical correlate applicable to all phylogenic groups has not been established yet.

In the case of warm-blooded animals capable of maintaining constant internal body temperature against external temperature variations (of course within a range and for a certain period of time), regulation of basal metabolic rate (as measured by tissue respiration) by thyroid hormones has been an accepted paradigm for thyroid hormone action. Their calorigenic action is well established. However, such an 'action paradigm' for thyroid hormones in the case of poikilotherms has been difficult to establish. Further, the diverse nature of their effects even within mammals has also created confusion in literature in early periods of endocrinology with regard to their classification into groups labelled as metabolic hormones and developmental hormones. They do bring about predominantly metabolic effects in mammals but their cognate receptors have been localized in nuclei of target tissue cells. This is similar to those of steroid hormones which are clearly classified as developmental hormones. Fittingly, thyroid hormones do have a role in brain development and severe thyroid deficiency leading to growth retardation is an established fact. Further, thyroid hormone T_4 has been long back established as capable of accelerating amphibian metamorphosis.

Notwithstanding all this, whether in poikilotherms, thyroid hormones have positive metabolic effects, whether they have only non-metabolic effects (e.g. growth and development) or whether other hormones can substitute for them is not clearly investigated and understood. Couple of years back Samir Bhattacharya and his group had elegantly demonstrated the unambiguous role of thyroid hormones in fish reproduction. Now Gupta and Mahanta (**page 326**) have shown that testosterone can function like the thyroid hormone T_4. They are demonstrating positive *in vitro* and *in vivo* effects of the androgen on peripheral tissue respiration.

The judicious use of antihormones (goitrogens and cyproterone acetate) in establishing the role of thyroid and gonadal hormones in calorigenic action *per se* in poikilotherms as well as in demonstrating the ability of thyroid hormone to potentiate the non-shivering calorigenic effect of catecholamines is noteworthy. In these animals, exogenously administered T_4 and T_4 have produced equivocal results in other workers' laboratories.

Hibernation among all phylogenic groups is associated with low metabolic rate. In hibernating mammals, thyroid tends to be inactive during winter months. On the other hand, thyroid exhibits moderate activity in winter months in poikilotherms. Gupta and Mahanta also demonstrate the differential sensitivity of hibernating and non-hibernating frogs to thyroid hormones and the ability of gonadal androgen to substitute for thyroid hormone in both types of poikilotherms.

K. Muralidhar

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CURRENT SCIENCE

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CORRESPONDENCE

Science and industry

I found the editorial of the 25 January 1996 issue particularly intriguing and significant. The editors are clearly pointing the journal in the right direction – toward greater contact with industry. In the present world climate for science, it would be suicidal to do anything else. I do not believe that the readers of *Current Science* have been sufficiently exposed to the science – the evolution – of current thought on the value of science not connected to industry and applications. Unknown to most, the model of post World War II American 'pure' or 'basic' science – colonized by the lure of money from the public purse, and mesmerized by the big gadgets of big science – had wreaked havoc on the real purity of 'pure' science. Very few scientists like Tennyson's Ulysses chase 'knowledge like a sinking star'. They are almost totally constrained by money, by fads, by peers, by bureaucracy with necessarily 'political' agendas. As money becomes scarce, US academics, for the wrong reason, look to industry. The major correct reason for linking to industry is that is where interesting, exciting problems – the interface with unexplained manifestations of nature appear. The other reason, of course, is that it is a way to serve the public good, since it is public money we use.

However, paragraph 2 of the editorial is simply wrong. It is the misperception foisted on to the public by the American academics. University–industry interactions are *very weak* compared to the total effort, and they contributed at best in a minuscule way to making these 'great economic powers'. I showed at great length in my book, *Lost at the Frontier* (ISI Press, 1985) and in subsequent papers how the government support of science 'ruined' the pre-war American *real* science where independent universities with modest help from the private sector really linked these two worlds. In chemistry, Speed Marvel and Roger Adams were great examples. In physics, contrary to the general view, Bell Labs was the mecca where *applications-driven* basic science was taken to Nobel heights. The transistor was driven by the most elementary and obvious need to eliminate the filament current drain. It did not originate in quantum mechanical calculations of energy levels. The post World War II government entitlement programmes for academic research while winning lots of Nobel Prizes in abstract science (at some billions of dollars of public money for each) *severed* the university–industry ties.

What Indian scientists should learn is that US (and worldwide) major industries between 1991 and 1993 made the discovery that there is no R.O.I. on basic research. The bogus self-serving economic analyses by academics to the contrary, *basic (undirected) research not connected to a product can never pay to the funder*. (This is a precise integrated statement which should not be truncated at any point, if attributed to me.) The proof is absolute and simple. If 100% of all major corporations that practised the opposite for 40 years (lavishly funding such) have realized their mistake, how can an academic analyst prove that they are wrong. If a company cannot make basic research lab–development lab interaction pay, how can any reasonable scientist claim that the threadbare connection between the results of a lone university researcher be effectively coupled to any one of a thousand companies anywhere in the world.

Indeed the logic of this argument can take us much further – toward 'privatization' (with all sorts of incentives) of *all* undirected truly basic research. Lacking space I can only refer the reader to Cambridge biochemist Terence Kealey's just published book *The Economics of Scientific Research* (St. Martin Press, 1997). It is an absolutely essential resource for anyone thinking about government and industry support of university research. Kealey has marshalled facts and arguments which will dominate the discussion for many years. The core of Britain (and Germany's) basic science was funded in its greatest years by private philanthropy.

I write not as an opponent but as a strong, but highly disciplined advocate of such university–industry (U–I) coupling. MIT and Penn state are and have been neck and neck in the competition for the leading university position in U–I coupling, with nearly $50 million/yr involved. And in that, the Materials Research Lab I started and directed for a quarter of a century remains the outstanding player.

The salient new points for Indian thinking about U–I coupling in today's climate are:

1. The dollar amounts are much smaller than in government grants (factor of 5 to 10).
2. The necessary response times are much shorter (factor of 5).
3. The coupling partners are in globalized setting so that US companies can couple to Indian universities and Indian or Japanese companies can couple with US or European universities. (Our lab does this very effectively today.)
4. Doing this kind of quick response research aimed typically at maximizing the functions of 'cheaper, faster, or greener,' not necessarily performance only, requires a certain mindset not very common among (American) academics.

Hence I subscribe enthusiastically to your editorial's last paragraph: 'it *will* enrich the Indian scientific scene'. But I add to your need for 'imagination and drive' the willingness to change. Perhaps Indian science could learn the lesson (totally ignored by post World War II American science), which Albert Einstein put to a Caltech adulatory crowd in 1931: 'It is not enough that you should understand about applied science in order that your work may increase man's blessings. Concern for man himself and his fate must always form the chief interest of all technical endeavors, concern for the great unsolved problems of the organization of labor and the distribution of goods – in order that the creations of our minds shall be a blessing and not a curse to mankind. Never forget this in the midst of your diagrams and equations.'

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Flawed policy of DST and DoE

I wish to state that the statement about DST imposing two draconian conditions on S&T projects submitted to them is not true (P. N. Andhare, *Curr. Sci.*, 1997, **72**, 158–159). As far as support for basic Science and Engineering Research is concerned, the scientific merit is the only ground on which funds are provided. Only on projects of applied nature, it is the policy to nurture linkages between research community and industry, and preference is given to projects involving industrial participation. During the year 1995–96, Science and Engineering Research Council has supported over 300 projects and spent Rs 37 crores approximately and scientific merit has been the only criterion in supporting these projects.

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P. N. Andhare accuses the DST and DoE of imposing 'draconian conditions that they nip in the bud *any* S&T proposal even before its evaluation on scientific merit' (emphasis added). The two conditions are (paraphrasing) (i) every proposal must have financial support from industry, and (ii) industry must give an undertaking in advance to productionize the R&D resulting from the proposal. In my capacity as the Chairman of the Programme Advisory Committee on Robotics and Manufacturing of the Department of Science and Technology, I would like to make it clear that the above-stated requirements are *not* preconditions for a proposal to be funded by DST.

Let me begin by giving a few statistics: The PAC-RM (and its predecessor, the PAC on Manufacturing Technology) have over the years made it a policy to nurture interaction between the research community and industry. Since 1991, the PAC has funded (after approval by SERC) a total of 39 projects at an outlay of Rs 512 lakhs. Of these, 18 projects have received partial funding from industry and other agencies to the tune of Rs 178 lakhs. Thus, the industrial support has been approximately one-third of the total funding. On the other hand, it can be seen that the majority of projects funded by PAC-RM have *not* received any industrial funding. Thus, Andhare is not correct in stating that 'any' project must have industrial funding in order to be considered. The statistics above (from a PAC that is among the most 'practical' in DST) bear this out.

Speaking as an individual, I would say that in order to qualify for funding, a research project must *either* consist of top-quality basic research, *or* address a problem of relevance to industry. In the former case, the criteria for assessing a project are the familiar ones, namely the past record of the researchers, the likelihood of the outcome of the research being published in top-quality journals, and so on. In the latter case, a major criterion for judging the relevance of a project to Indian industry must surely be the willingness of the industry to underwrite the cost of the project. There is nothing unreasonable about this criterion.

Unfortunately what happens far too often in our country is that people try to pass off as 'practical' projects whose only notable feature is a total absence of any novel theoretical ideas. Thus the operative presumptions seems to be that if a project will not contribute to basic research, it must therefore be deemed to be 'practical'. In such situations, I see nothing wrong in calling the bluff of such proposers by asking them which industry is interested in their work, and why such industries are not paying at least a part of the cost of the project.

Let me repeat that if a person is doing basic research work at an internationally competitive level, then industrial participation is not called for at all. On the other hand, stringent steps must be taken to guard against persons trying to pass off third-rate theoretical or 'applied' work as 'practical' R&D.

Finally, I cannot agree with Andhare's railing against 'market' forces in S&T. Whether he acknowledges it or not, there are *always* 'market' forces at work in every arena, not excepting S&T. Even carrying out 'basic' research is subject to 'market' forces. Why else do new ideas suddenly become 'hot' while others becomes 'cold', even in purely theoretical subjects such as mathematics? In order to publish a paper, even in purely theoretical subjects, it is necessary to make a substantial contribution *on a topic in which the research community is interested.* No journal will publish a paper on a topic that is deemed by the community to be outdated or not of interest to anyone. Unfortunately what some persons want in our country is not academic freedom, but rather freedom from accountability. But that would be licence and not freedom.

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Essentials of Nuclear Chemistry: Critique of a review

P. S. Goel's comments on my book (*Curr. Sci.*, 1996, **71**, 155–156) can be grouped under six heads: (1) Quoting unupdated values of some constants, (2) Conceptual errors, (3) Irrelevant references, (4) Mathematical errors (only two), (5) Use of outdated cosmological models, and (6) Language problems.

While the values of fundamental constants have been determined with high precision and are universally accepted, there are some of limited applicability whose values are known with less accuracy. Under this category come the proton decay constant, the ^{14}C content of living matter, the ages of the oldest rocks and of the earth. In fact, proton decay as a distinct form of radioactivity is yet to be accorded international recognition. This value, quoted in a footnote in my book, was taken from a popular lecture by an eminent scientist in the Indian Science Congress session of 1982. Since then there has been no new information on the subject. The approximate value quoted by me, viz. one proton out of 10^{30} per month was only to indicate the great rarity of proton decay. Similarly, all the workers on dating by ^{14}C are not agreed on the exact content of this isotope in living organic matter. Values cited vary from 13.6 to 16 c min^{-1} g^{-1}. I have only stated this fact in my book. Similar remarks apply to the assessment by different workers of the age of the oldest rocks of the world and of the age of the earth based on $^{40}Ar/^{40}K$ ratio. Stating these facts cannot imply lack of updating.

If I have referred to Urey's work in this context, it is because the famous geologist K. Rankama too had referred to Urey, not once, but some 30 times, in his classic work, *Isotope Geology* (McGraw-Hill, 1954). It is not clear how my referring to the Nobel laureate's work can be considered 'blatant misinformation'. My description of the term 'nuclide' as a given combination of *Z* protons and *N* neutrons in the nucleus, without referring to *Z* electrons in the outer sphere, and of 'isobars' as nuclides with a constant nucleon number A ($\equiv Z+N$), are succinct and error-free. Yet these are cited as examples of conceptional errors without pointing the error. Similarly, the numbers of stable odd *A* nuclides, 50 with Z-odd and 55 with *N*-odd being nearly the same, show *the charge-independent nature of the stability of odd-A nuclides.* This statement is also considered as a conceptual error. Similarly, the review includes other statements of mine as involving conceptual errors, without the errors being pointed out.

Goel has rightly pointed out two grave mathematical errors, (i) p. 134, last line, the expression $e^{(\lambda_1-\lambda_2)}$ should read $e^{(\lambda_1-\lambda_2)t}$. The correct expression is used in the table on p. 135 and elsewhere. (ii) The second error refers to the calculation of the surface area of 1 g of barium sulphate precipitate (p. 383, line 13), the formula weight of $BaSO_4$ is 233.4 and not 223 as given. This leads to a value of 19.51×10^{-16} cm^2 g^{-1} instead of 4.11 cm^2 g^{-1} as pointed. I am grateful to Goel for pointing out these two errors. These will be rectified at the time of the next reprint.

It is unfortunate that Goel did not see a single positive point in the 500-page fourth edition. The book has been reviewed by some others as well and it is widely used in the country and outside and has been translated into the Serbo-Croatian by the Faculty of the University of Belgrade and into Iranian by the Iranian Atomic Energy Organization.

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Science policy in neo-liberal India: Corporate culture, basic science and scientific credibility*

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In the present neo-liberal era, there is great pressure to introduce corporate culture in managing Indian science and scientists and to de-emphasize basic science in favour of developing immediately useful technologies. This is ironic because the performance of our better universities and R&D institutions has been much closer to international standards than that of our corporate sector. With very few exceptions, the latter has been almost inimical to indigenous R&D. Nearly all serious scientists have worked in public-funded institutions. The demands of corporate culture are likely to accelerate the flight of bright youngsters from scientific careers and even reduce the credibility of science.

Introduction

The Science Policy Resolution adopted by the Parliament on 4 March 1958 stated that the aims of the scientific policy of the Government of India would be:

(i) to foster, promote and sustain, by all appropriate means, the cultivation of science and scientific research in all its aspects – pure, applied and educational;

(ii) to ensure an adequate supply, within the country, of research scientists of the highest quality, and to recognize their work as an important component of the strength of the nation;

(iii) to encourage and initiate, with all possible speed, programmes for the training of scientific and technical personnel

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*Based on a lecture delivered by the author on 24 January 1997 at a seminar on 'Science policy and scientific temper', organized by the Dept of Civics and History, University of Bombay.

on a scale adequate to fulfil the country's needs in science and education, in agriculture and industry, and defence;
(iv) to ensure that the creative talent of men and women is encouraged and finds full scope in scientific activity;
(v) to encourage individual initiative for the acquisition and dissemination of knowledge and for the discovery of new knowledge, in an atmosphere of academic freedom;
(vi) and, in general, to secure for the people of the country all the benefits that can accrue from the acquisition and application of scientific knowledge.

The SPR further stated that: 'the Government of India has decided to pursue and accomplish these aims by offering them an *honoured position*, by associating scientists with the formulation of policies and by taking such other measures as may be deemed necessary from time to time'.

The preamble to the resolution recognized that 'it (science) has not only altered man's material environment, but, what is of still deeper significance, it has provided a new tool to thoughts and has expanded man's mental horizon'.

The major moving thought behind this resolution was the realization that of the three main factors, namely technology, raw materials and capital that hold the key to national prosperity, technology was the most important. However, it also asserted that 'it is an inherent obligation of a great country like India, with its tradition of scholarship and original thinking and its great cultural heritage, to participate fully in the march of science, which is probably *mankind's greatest enterprise today*'.

It is now nearly four decades since the adoption of the above resolution. India is in the 50th year of its independence and the world is on the threshold of the 21st century. Indian science is in turmoil, and its public image is not very bright. Serious scientists are under great pressure to accept the culture of the corporate sector which in the Indian context has contributed little to R&D and produced hardly any goods or services of international standard. And most, including those who ought to know better, are succumbing to these pressures. Even the best scientific institutions are increasingly becoming unpleasant places to work, with a crude authoritarianism creeping in and academic freedom and scientific values not receiving the importance they deserve. Scientifically tainted individuals can today receive both protection and power merely by promising relevance (often the last resort of the mediocre) or ECF (external cash flow). Managers of science enjoy great power within their institutions but little credibility outside. Bright youngsters increasingly consider a career in basic science an unattractive proposition. Though many still flock to engineering, their final destination is mostly marketing and management and rarely ever development of advanced technology.

It is certainly an apt time for stocktaking. What has gone wrong? Has the performance of Indian science been really as bad as its reputation in certain quarters in this country would indicate? Were the dreams of the Indian architects of the SPR (Nehru and Homi Bhabha) unrealistic or unjustified? Have the policies enunciated in SPR become irrelevant now? If not, then why are scientists being forced to act against these policy directives? Has Indian science been wrongly maligned by the sustained campaign of anti-science ideologues and vested interests? Is it suffering because of the sins of its own managers and/or due to intellectual laziness and timidity of its foot soldiers? Or is the corporate sector, which has shown little inclination to invest in R&D, trying to hijack the S&T infrastructure and competence diligently created in the public sector by an enlightened government? How do the public investments in science compare with material returns that society has received from this investment? What is the nexus between developing S&T infrastructure and scientific temper? How much of the current pressures on Indian science are due to Indian people's disenchantment with science and how much of these are merely due to internal contradictions between basic scientists and technologists, and between power hungry careerists and serious scientists? How much of these are due to international trends and fashions? How much has our official scientific enterprise contributed to the development of scientific temper among our people? What role have the NGOs played in this regard? What more can the latter do? Is it desirable, or even safe, to leave the making and implementation of science policy totally in the hands of scientists and technologists who may not have had the time or inclination to educate themselves in the related sociological, philosophical, historical and intellectual issues? Is there a need for writing a critical history of the development of Indian science in recent decades? If yes, who should write it? Should it be scientists who have watched the working of our R&D institutions and science departments as insiders? Or should it be professionally trained historians with science education? Those who have enjoyed enormous power in Indian science must be subjected to historical accountability. But how are we going to do that?

These and many more questions need to be asked by our science policy makers, those charged with implementation of these policies, the working scientists and concerned individuals in other fields of intellectual activity. Here I shall try to give my views on some of these questions. These are entirely personal views of someone who entered a scientific career just a few years after the SPR was adopted and who has been dismayed in recent years by steady attacks on the achievements of Indian science both from within and outside and the resultant decline in the attractiveness of science as a career. These are also the views of someone who is concerned that the recent emphasis on introducing corporate culture into Indian science is likely to further drive away bright youngsters from science leading to decline in its performance which is unlikely to help the country in the long run. Perhaps even more importantly, the introduction of corporate culture may reduce the credibility of scientists and science, built by a scientific culture which demands much higher levels of objectivity and integrity than found in any profit-oriented, pragmatic culture.

Performance of Indian science

There is a general belief in this country that Indian science has not done well in the post-Independence era. Usually, three facts are cited to support this view. First, in recent years we have not produced outstanding scientists of the eminence of Raman, Bose and Saha, as highlighted in a recent *Times of India* article by J. Narlikar. Second, Indian S&T has not made significant contributions towards developing technologies for our industry. And, third, in spite of investments in S&T, we have not been able to solve our problems of poverty and underdevelopment. On all the three counts the criticism is less justified than it appears at the first sight. This point needs to be explained and emphasized as the constant criticism, especially when it comes from quarters which have no anti-science bias, can reduce public support for S&T and make science less attractive as a career option for brighter students. The consequences of this can be serious for our national image and development in the long run.

Narlikar considers the neglect of our university system, the parochial political considerations in the running of our universities, and the failure of the research institutes to have teaching programmes and attract young students as some of the main causes of our failure to produce scientific stars. All these are valid criticisms and corrective action should help. However, there is another side to the story. The performance of our universities and national research institutes has not been really as poor as most of us seem to believe. It is true that both in terms of production of scientific stars and developing novel technologies, our record compares unfavourably with that of the more advanced countries, especially the USA, with which we seem all the time to compare ourselves. But this is not a fair comparison, given the relative levels of our investments in science and the general level of our economic, industrial and socio-political development. It is more realistic and revealing to compare the records of our science education and R&D sectors with those of other sectors of our national life. While almost nothing produced by our private sector (be it a cake of soap, a pair of shoes or trousers, a news magazine, or a radio set) could be sold in the supermarkets of advanced nations until recently, the best products of our universities and research institutes, (viz. the students, scientists and research papers) have been of international standards and have sold in the most competitive global markets. Is there any other field outside our higher education and R&D system (with probable exceptions in the areas of purely Indian culture, such as classical music) which can make such a claim?

It is not insignificant that in the present liberalization the one factor which most international investors find attractive about this country is the abundance of highly skilled technical manpower. If today many advanced countries are trying to stop the entry of Indian scientists and technologists into their countries because they successfully compete with their own citizens for the best paid jobs, surely for this state of affairs some credit has to be given to what Indian universities and scientific institutes have been doing. This is perhaps a better index of good performance of post-independence Indian science than even the few memberships of the International Academies and invitations to Plenary Lectures (as pointed out by Narlikar), though I have absolutely no intention of belittling the latter honours earned against heavy odds. Certainly, the achievements of India-trained scientists and technologists in the post-independent era have done more than did Ramans, Sahas and Boses in changing the image of the country from an abode of *sadhus* and snake charmers to that of a major reservoir of scientific and technological personnel. Today, one hardly hears of the Hindu (read irrational) mind, which was a common phrase in western discussion of India up to the fifties and sixties.

Ramans, Sahas and Boses remain important role models and sources of national pride to India. But it should be recognized that the world in which they operated was very different from the post war world available to the later Indian scientists. It should be remembered that during the last few decades even the more advanced nations of western Europe have lost large number of elite scientists and brightest students to the US which has provided a working environment and facilities not available elsewhere.

Impressed by its experience during the second World War when numerous expatriate scientists from Europe played key roles in the Manhattan project and elsewhere, the US, as a matter of policy, provided a hospitable and attractive environment to scientists, technologists and bright students from all parts of the globe. Government support for research expanded rapidly. Professors received funds to hire large teams of best available graduate students and post-doctoral fellows from anywhere in the world. In this they succeeded admirably because the research facilities, the liberal intellectual environment, and the salaries available in the US were too irresistible for most students and scientists elsewhere in the world. Many pieces of equipment, chemicals, etc., which were earlier made by scientists themselves, became available commercially which gave a big advantage to those with more money and proximity to the centres of production. Little wonder that suddenly most of the star scientists were to be found in the US. Not only did India's Chandrasekhar, Khorana, Inder Verma, and Ananda Chakravarty choose to emigrate to the US, so did some of the best scientists from most other major nations. If a young Raman or Saha in post-independence India had resisted the temptation to emigrate to the US and doggedly stayed on in India, he would be functioning under considerably greater relative handicaps than he faced in the earlier era. If he had continued to work on exciting contemporary problems, he would have had many more competitors working on the same problems and supported by better facilities, brighter students and better trained post-doctoral fellows. The chances of his being scooped would be quite high. In the first past-the-post system of scientific credit, Raman and Saha might have received no credit for the work done by them.

Under such circumstances, it is indeed remarkable that India has retained sufficiently large number of good scientists to keep the standards reasonably high. The important point here is that our failure to retain some of our best students and scientists is less due to the manner in which our universities and research institutes have functioned and more due to our socio-economic conditions and policies originating elsewhere, about which we could have done nothing.

As to the question of our record in developing technologies for our industry, I think the reason lies again in the state of our economic development. Our industrialists, with limited capital from international standards and a vast captive market, have behaved quite rationally in acquiring proven, even if dated, technologies from elsewhere and using them to manufacture goods for our needs which Indian consumers accepted readily. To expect our industrialists to compete with the multinationals in R&D, or to use only the (unproven) technologies developed in Indian research laboratories, would require them to be financially imprudent. It is, therefore, not surprising that even though during the last 50 years most of the CSIR laboratories have concentrated on applied research (it is a mistake to think, as some seem to do, that the stress on industrial research in CSIR laboratories is a new phenomenon), few of its technologies have been used by Indian industry. Even in the future only such technologies developed by the CSIR are likely to see the light of the day as are sold to the major (mostly multinational) companies with in-house R&D capabilities to develop them into marketable products. It is unrealistic to expect Indian scientists and technologists to compete with international industrial houses in generating marketable technologies of comparable standards, without even the benefit of interacting with the user industries.

In advanced countries, scientists and technologists in publicly funded R&D institutes only generate technology ideas which are then developed into technologies and products by industrial scientists. Under the best of circumstances, scientists in Indian government institutions could be expected to compete with their counterparts elsewhere, not with the researchers working in industry. The main point is that though Indian industry has its own reasons for not using the technologies developed in public funded Indian research laboratories, as well as for not investing in in-house R&D of its own when it could easily buy ready-made technologies of standards acceptable to the Indian consumers, these reasons were not good enough to hold Indian scientists exclusively responsible for not developing and transferring technologies to the Indian industry. Indian research laboratories could have succeeded in transferring technologies to Indian industry only if the latter had no access to the proven technologies in the international market. This, of course, would not happen in non-strategic areas. International industry would not like its potential competitors to grow, if all that was required to stop it was to offer its obsolete technologies, and may be a few foreign trips, to the Indian industrial managers.

It is noteworthy here that in strategic areas such as atomic energy, defense and space, where the advanced nations were not willing to part with their technologies, Indian scientists and engineers have a much better record of the utilization of their efforts, even though the unavoidable teething troubles may have made these developments relatively slow.

Coming to the question of the contributions of Indian R&D to poverty removal and development, firstly it must be recognized that these contributions have not been insignificant. If we have not had a famine for the last 30 years and a war for the last 25 years and if our life expectancy has improved substantially, some credit for it does go to our S&T sector. Even if the contribution of agricultural scientists to the increased food grain production (approximately 140 million tonnes per year in the post-independent era) is calculated at Rs 1/kg (i.e. about 20% of the actual cost), this works out to a staggering Rs 1,400 crores per year, certainly much more than our total annual expenditure on R&D. Taken as a whole, Indian R&D is clearly more than paying its way. Our poverty and underdevelopment certainly remain. But to think that these problems will be solved faster by further reducing our already meagre investments in R&D, or by introducing corporate culture into our publicly funded R&D institutions is unrealistic. Such steps can only destroy what has been achieved, make scientific careers still more unattractive, and further aggravate our problems in the future. Our real problems lie in lack of capital and an ineffective population policy, especially the latter, on which we are silent these days.

Useful technologies vs basic research

Our tendency to be over-critical of ourselves and failure to appreciate that we have been doing something right about our higher education and S&T, has created an environment in which there is a real danger that much of the past good work may be undone. I refer here to the excessive emphasis in the last few years on support of only immediately useful S&T. Not only are the national laboratories being pressurized to de-emphasize basic science, which is absolutely essential to maintain high standards, but even the universities are being exhorted to become handmaidens of industry which, understandably, would support only short-term projects with prospects of immediate profits.

It is pertinent to note that the emphasis on immediately useful science and reduced support for basic science is not an original Indian idea. This is an imitation of what has become a fashion in several advanced countries since the era of Reagan and Thatcher. This is also true of the exhortations to the scientists to establish linkages with industry. This development has disturbed several of the more thinking scientists and intellectuals in the West who have articulated the risks involved in this approach. For instance, in a recent letter to the *Science* (16 August 1996), Nobel Laureate Arthur Kornberg pointed out that the antibiotic penicillin, as well as the entire molecular biology leading to the Human Genome Project (HGP) and bio-technology, would not have been possible, but for the support to the relatively unplanned, curiosity-driven research. At the same time, according to him, the high level of planned support to anti-cancer research has yielded much poorer dividends, because the knowledge generated to solve the problem simply did not exist.

Several other points which have recently appeared in international literature are of relevance in the context. A study conducted by the Science Policy Research Unit at the University of Sussex in the UK, has concluded that the economic impact of basic research has to be measured not only in terms of the value of the knowledge that it generates, but also in terms of producing the trained manpower which can make use of such knowledge, even if it is generated elsewhere. This is extremely important in our case. To cite an example, benefits emanating from the HGP are not going to be finally utilized by the needy countries if there is no trained manpower contributing to the generation of this knowledge. Hence, India would be making full use of knowledge generated by the HGP only if it invests money in projects aimed at generating knowledge in this area, even if this knowledge may not bring us glory that the advanced nations with their much bigger investments will corner. India will at least generate manpower needed for using the internationally available knowledge in patient care, in reducing the genetic load in our population, and in utilizing the technologies spun off by this project in agriculture, animal husbandry and molecular anthropology. The architects of our SPR, much more realistic as they were than some of our present-day critics and managers, would have been satisfied with the above as our initial goal.

In a beautifully argued article (*Interdiscip. Sci. Rev.*, 1995, **20**, 281–288), reproduced in *Current Science* (1996, **71**, 148), Martin Rees points out that a panel set up by the National Academy of Sciences, USA, in 1937 to predict the technologies of the following few decades, did *not* include among its predictions the nuclear power, transistors, antibiotics, jet aircraft, rocketry, or computers. All these developments were possible because in the post-war era the US government invested heavily in supporting curiosity-driven, investigator-initiated research projects in areas of *no obvious* immediate benefits. In contrast, the USSR which greatly emphasized the utilitarian science over basic science, contributed relatively much less to the development of novel technologies which have changed the world today. (It is, perhaps, not irrelevant to refer here to T. D. Lysenko who emphasized the empirical agricultural technology like vernalization and used his friendship with Stalin to decimate the growth of genetics in the USSR; the consequent failure of the USSR agriculture to grow food crops at the required levels in the long run played a key role in the failure of the entire Soviet experiment.)

Black holes and dinosaurs

Another interesting point made by Rees in his essay is that the common people have maximum interest in some of the areas of science with hardly any conceivable use. Cosmology and dinosaurs are much more likely to make front-page news than any of the more useful technological breakthroughs. This supports a feeling I have always had that most of the attacks on basic science really do not come from general public but are generated by the internal politics within the scientific community. Rees also points out that a football team in which each player has to score goals in order to retain his place is unlikely to be a functional outfit. This should be a sobering thought for those, including some very influential policy makers, who would like to support only such basic researchers the impact of whose research is visible to all. It is essential to maintain a broad science base and a web of connections between different disciplines.

Threat to credibility of science

John Ziman, Emeritus Professor of Physics at the University of Bristol, pointed out in his 1995 Medawar lecture that in the current emphasis everywhere on useful research what is at stake is the very 'objectivity' of science. Scientists who are employed to do useful science will clearly not be in a position to publish their findings if they go against the interests of their employers. The implication of this is that the credibility of science and scientists will take a serious beating. It was the objectivity of science and the relative, even though not absolute, disinterest of the scientists in the benefits of their work that gave science a unique prestige as a methodology of generating knowledge. Science as a cultural and intellectual activity will lose its credibility, and in the long run will also lose its ability to benefit society and generate knowledge and technology, if public funding and prestige is denied to those who are willing to work in intellectually challenging areas even if the potential applications of their work are not apparent in the beginning.

Lastly, it is important not to ignore the lessons of history. Two examples, one of a long-term and the other of short-term historical context should suffice to make my point. (i) During the centuries immediately preceding the British colonization, India was ruled by one of the most powerful empires – the Mogul empire – in history. During this period, India had acquired, developed and used a large number of useful technologies in diverse areas such as production of textiles, fancy gold and silver embroidery, metal work, architecture, traditional medicine, perfumes, sophisticated musical instruments, etc. The country was sufficiently wealthy to attract traders from far away lands. However, little attention was paid to keeping track of the advances in S&T taking place elsewhere and to making original contributions to the advancement of knowledge in these areas. The result was that European traders could extract large concessions from Indian emperors simply by holding out the threat of attacking the Haj ships on the high seas to which Indians had no answer. Perhaps, even more importantly, the achievements of the Europeans in S&T so dazzled the Indian intelligentsia that many readily supported their rule, which was certainly an important factor in the easy colonization of India by Britain. (ii) The Government of India had created two Research Councils (viz. ICAR and ICMR) in the area of applied biology. The two have made major contributions toward improving our food production and health standards respectively. However, they made the mistake of concentrating almost exclusively on immediately useful projects and paid little attention to developing expertise in basic biology on which their future performance would depend. The result was that when in the 1980s the conceptual framework earlier developed by molecular biology altered the technological basis of modern agriculture and medicine, the ICAR and the ICMR had insufficient competence to exploit the new technologies. Nearly all the funding for molecular agriculture and molecular medicine had to come from the newly created Department of Biotechnology, and most work in these areas had to be done in the Institutions outside the purview of ICAR and ICMR. One shudders to think as to what would have happened to basic as well as applied biology in this country if the Government of India had not either created the DBT or if this Department had not been lucky to have an enlightened leadership which recognized the importance of basic science in developing technology. One hopes that other agencies which are keen to deemphasize basic research would learn the lessons from our earlier experiences.

To summarize, a realistic assessment in the light of the SPR shows that the performance of the Indian S&T sector, especially the R&D institutions and universities, which cultivated all aspects of science (pure, applied and educational), and gave reasonable encouragement to creative talent and individual initiative in generation of new knowledge in an atmosphere of academic freedom, has not been as bad as is generally believed in this country. They have succeeded in producing research scientists of international standard and in training large numbers of scientific and technical personnel who are a major source of our strength as a nation today and who can help in rapid industrialization of the nation, given adequate capital and proper economic and industrial policies. These institutions and basic research need continuous support. Our emphasis on immediately useful research at the expense of basic science can kill the goose that lays the golden eggs.

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Fertilization in flowering plants – What is new?

K. R. Shivanna and N. S. Rangaswamy

The process of fertilization is more complex in flowering plants than in other groups of plants. Following successful pollination, a pollen grain germinates on the stigma and the resulting pollen tube grows through the stigma and style and carries the two male gametes into the ovule. Eventually the pollen tube enters the embryo sac and releases the male gametes. One of the male gametes fuses with the egg to form the zygote and the other with the central cell to produce the endosperm, thus completing the process of double fertilization, a unique feature of flowering plants. The zygote gives rise to the embryo; the central cell-derived endosperm nourishes the embryo. Thus, fertilization in flowering plants takes place deep inside the ovule and imposes technical problems for basic studies as well as for effective experimentation. Because of this constraint, our knowledge of fertilization in flowering plants has been rather meagre and lags far behind that in animals and some lower plants in which fertilization is external and can be observed under the microscope. One of the main objectives of the experimental embryologist has been to achieve fertilization *in vitro* by using isolated egg and sperm cells, and development of the *in vitro* formed zygote into embryo. This would enable effective experimentation on fertilization in flowering plants comparable to that in animals and lower plants.

A beginning on these lines was made over 30 years ago in the laboratory of the late Panchanan Maheshwari at the University of Delhi. Maheshwari and his associates[1] cultured isolated groups of ovules and pollen grains of opium poppy (*Papaver somniferum*) together on a nutrient medium. Pollen germination, pollen tube entry into ovules and double fertilization proceeded normally; the fertilized ovules developed into viable seeds; this research report in *Nature* was covered in leading newspapers in India and abroad. Subsequently the technique was refined to minimize injury to the ovules and to overcome self-incompatibility[2]. Although the technique has been successfully used over the years to overcome crossability barriers in several species[3,4], progress in advancing the technique to achieve *in vitro* fertilization with isolated egg and sperm cells was hampered for want of techniques to isolate viable male gametes (sperms) and eggs and fuse them *in vitro* to achieve fertilization.

Rapid advances in protoplast technology and somatic hybridization during the 1970s and the 1980s gave new impetus to studies on *in vitro* fertilization. Recent progress in recombinant DNA technology, which made the egg, sperm and zygote very attractive systems for genetic transformation, compounded the interest in research on *in vitro* fertilization. Studies were initiated the world over on isolation of sperms and embryo sacs in the early 1980s and the progress was overwhelming[5]. Basically two procedures were employed to isolate sperms from pollen grains: mechanical method and osmotic shock method. In the first, the pollen grains are ruptured mechanically by grinding them in isolation medium, and in the second by incubating the pollen grains in a hypotonic solution. The debris is removed through filtration and the sperms are then separated through density gradient centrifugation. Embryo sacs are generally isolated by combining enzymic maceration of ovules with microdissection. Subsequently the technique was further refined to isolate protoplasts from constituent cells of the embryo sac (egg, synergids and central cell). Thus, by 1990, protocols were available for isolation of viable protoplasts from sperms and eggs of many plant species, and the stage was set to make serious attempts to achieve *in vitro* fertilization.

The pioneering success of fusing isolated egg and sperm cells *in vitro* was accomplished in maize by Kranz and his associates at the University of Hamburg in 1991 (ref. 6). It took another 2 years to standardize the conditions to realize embryogenesis and plantlet formation from *in vitro*-formed zygotes[7]. The fusion of gametes was performed under the microscope in microdroplets (2 μl) of fusion medium covered with mineral oil. Isolated egg and sperm cells held in microcapillaries were transferred to the fusion droplets by using a computer-controlled dispenser. The egg and sperm cells were aligned electrophoretically or mechanically with microneedles and fusion was induced by giving one or a few short pulses of direct current. The fusion was completed in <1 s and karyogamy occurred in <1 h from fusion. As in somatic protoplast fusion, in gametic fusion also the composition and osmolarity of the medium were critical. The *in vitro*-formed zygotes were cultured on a semipermeable membrane placed on fast growing nonmorphogenetic cell suspension cultures derived from maize embryo or microspores. In these nurse cultures the zygotes established polarity, showed as high as 85% division, and gave rise to globular structures, proembryos and transition phase embryos comparable to seed embryos *in vivo*, and eventually to fertile plants.

Next, *in vitro* fusion of isolated gametes in maize was achieved not with electric pulse but in the presence of high calcium[8] and high alkalinity (pH 11) (ref. 9); also, attempts were made to microinject sperm nuclei into isolated embryo sacs[10]. Furthermore, it has been possible to regenerate whole plants of maize by culturing the *in vivo*-formed zygote[11]. Recently sperm cell has been fused with the central cell also; the resultant fusion product was capable of growth comparable to endosperm[12]. A further milestone has been the demonstration that the fusion product of maize egg and sperm of the related genera, wheat and sorghum, underwent divisions, but not that of maize egg and sperm of the unrelated genus *Brassica*. Even more significant has been that the fusion product of two maize eggs failed to undergo divisions[11]. Most of the achievements of the past decade on *in vitro* fertilization have been with maize, and there is no compelling reason to disclaim that the various techniques employed will not work equally well with other higher plant species.

The advances on *in vitro* fertilization and embryogenesis have opened up a vista of experimental studies. Some of the fundamental problems that can be effectively researched on (using the technique of *in vitro* fertilization) are: (a) mechanisms of recognition, adhesion

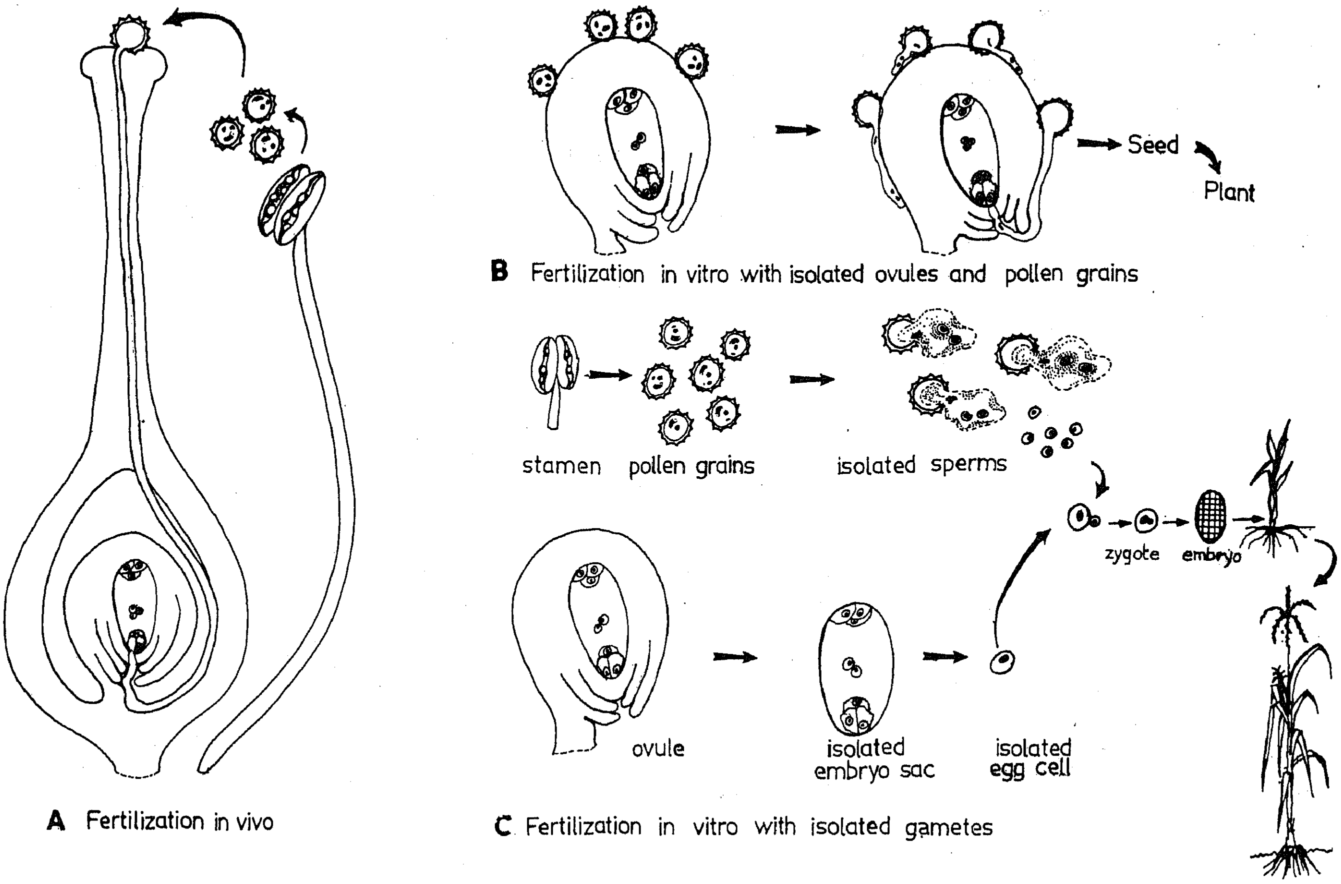

Figure 1. Diagrammatic representation of fertilization *in vivo* and *in vitro*.

and fusion of gametes, (b) parthenogenesis, (c) polyspermy, (d) establishment of polarity in the zygote, and (e) genetic regulation of early embryogenesis.

Indeed, *in vitro* fertilization and embryogenesis provide a very powerful biotechnological tool. Besides its potential application in overcoming incompatibility barriers, its application in genetic transformation studies using the sperm, or the egg, or the zygote is very promising. Studies have already been initiated on these lines; of late a paper from Duma's laboratory (Lyon, France[13]) describes optimal conditions for efficient microinjection of DNA into cultured maize zygotes and transient expression of the microinjected genes in the zygote. It is needless to emphasize that researches on fertilization in flowering plants have entered an exciting phase; in the coming years rapid progress not only in understanding the process but also in exploiting it for crop improvement will accrue, and indeed many a young plant scientist will find the work a much rewarding avocation. And who knows, *in vitro* fertilization may lead to raising a hybrid between the two groups of seed plants in not too distant a future!

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New light on the antiquity of Siwalik Great apes

Until now, fossil hominids in the Siwaliks of Indian subcontinent were known from Chinji–Dhokpathan successions ranging between 13.1 and 5.1 m.y. (ref. 1). We document here two isolated hominid teeth (coll. SSG), a sivapithecine molar and a dryopithecine incisor collected from a vertebrate fossil-bearing succession resting conformably over the Upper Murree exposed at Ramchand in Udhampur district, J&K (Figure 1). Ramchand is situated about 10 km southeast of the classic vertebrate fossil locality of Ramnagar from where many vertebrate collections including hominids have been reported earlier[2–4].

Though the newly discovered fossil material is scanty, the specimens hold considerable significance in view of the extreme rarity of hominid fossils in Siwaliks, and more for their provenance from a much older stratigraphic level than previously known from this region. The find has an important bearing on the question of hominid diversification and their global distribution and migration patterns during Early Miocene.

The isolated molar tooth (Figure 2 *a–c*) was extracted *quasi in situ* from a sandstone bed, southeast of Ramchand Ridge, resting 100 m above the Murree/Dodenal (= Kamlial) Member contact[5]. It has suffered damage as a result of which its posterior part is missing. However, on the basis of morphological details of its well-preserved anterior two cusps, viz. protoconid and metaconid, and the enamel folds, the molar is indisputably referable to the genus *Sivapithecus* Pilgrim. The specimen represents the lower left last molar and evidently belongs to a robust form intermediate between *Sivapithecus indicus* Piligrim and *S. giganteus* (Piligrim)[6,7].

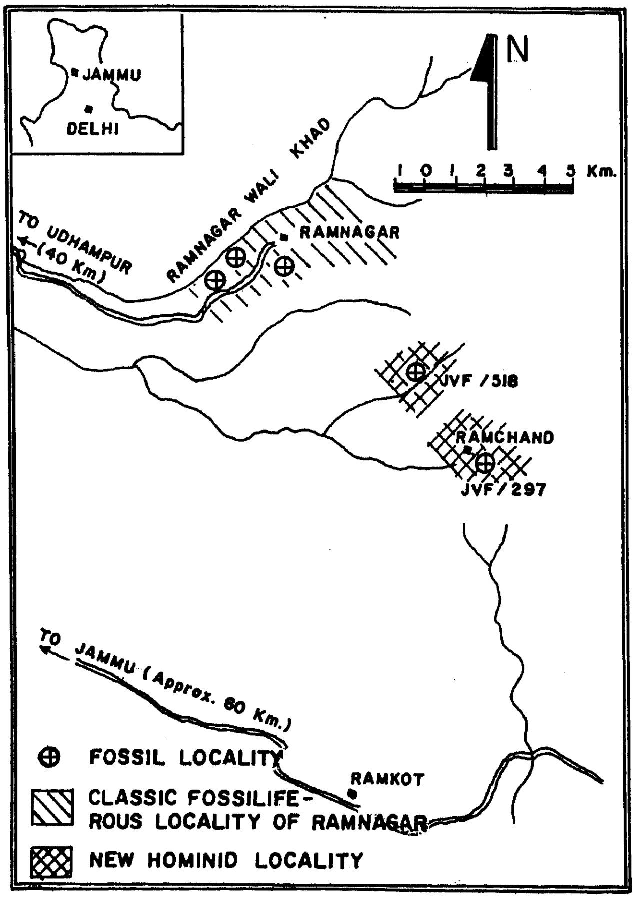

Figure 1. Map showing the classic fossiliferous locality of Ramnagar and the new hominid sites near Ramchand, Udhampur district, J&K.

The other specimen, an isolated upper incisor (Figure 2 *d, e*) was extracted from a sandstone layer resting stratigraphically about 600 m up in the same continuous succession west of Ramchand (Figure 3). The source bed falls in the gradational contact zone of Dodenal/Ramnagar (= Chinji) Member[5]. The enamel folds on the internal side and wear pattern of the crown suggest the tooth to be of a large dryopithecine. We have provisionally assigned it to the genus *Dryopithecus* Lartet.

The Ramchand–Ramnagar section exhibits an uninterrupted sequence of Upper Murree to Middle Siwalik and consists of an enormous pile (4000 m) of sand/siltstone, red clay/shale and mudstone alterations (Figure 3) with gentle dips (8–20°) to the northwest. Ramnagar vertebrate fossiliferous horizon is located near the top of this succession whereas the present *Sivapithecus* yielding horizon stratigraphically lies about 1500 m below the Ramnagar hominid level. The incisor tooth was found near the middle of this succession.

Associated fragmentary vertebrate fossils recovered from vicinity of the *Sivapithecus* site, within 200 m of the stratigraphic column above the Murree Lower Siwalik contact include forms such as: Siluridae indet., *Trionyx* sp., *Gavialis* sp., *Deinotherium* sp., *Gomphotherium* sp. cf. *G. angustidens*, *Aceratherium* sp., Suidae indet., Tragulidae indet., *Dorcabune* sp., *Dorcatherium* sp., Anthracotheriidae indet.

Numerous other fragmentary limb bones, isolated vertebrae, coprolite and scutes belonging to fishes, chelonia snakes, etc. also form part of the faunal assemblage recovered from the Ramchand hominid locality.

The Murree/Lower Siwalik contact, particularly the base of Kamlial has been found to occur near the top of Chron 17 (18.3 m.y.) in Kamlial/Chinji stratotype in Pakistan[8], which is about 200 km from Ramchand as the crow flies. Since the Murree/Lower Siwalik lithologies show lateral continuity over large distances, we presume approximately the same age for our *Sivapithecus* molar specimen and aforelisted fauna. The assemblage indi-

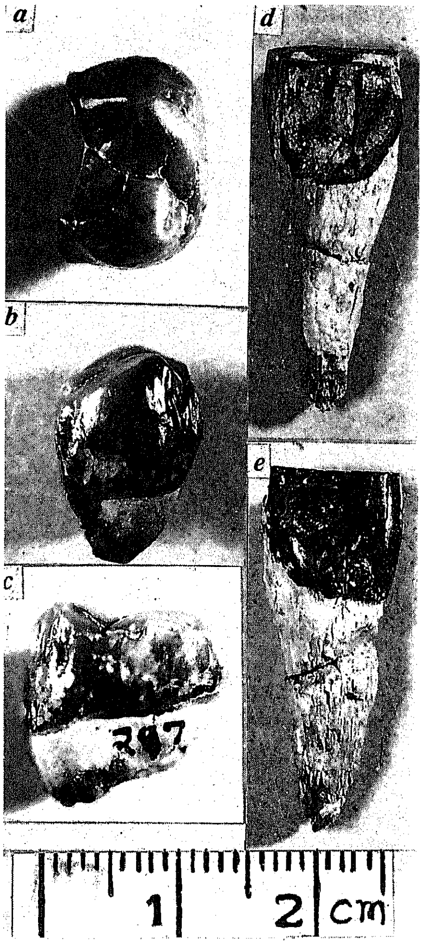

Figure 2. Hominid teeth from Ramchand *Sivapithecus* sp. (No. JVF/297), last lower left molar showing crown (*a*), lingual (*b*) and anterior (*c*) views. *Dryopithecus* sp. (No. JVF/518), upper incisor tooth showing internal (*d*) and external (*e*) views.

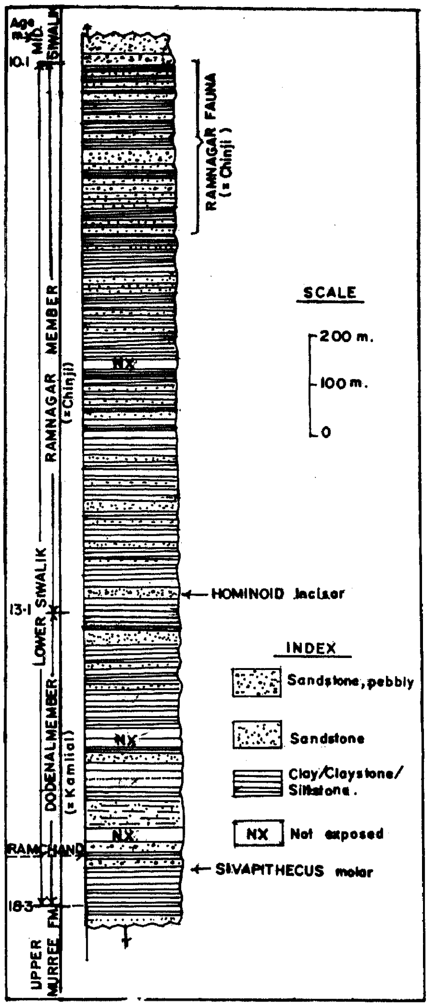

Figure 3. Lithocolumn along Ramchand–Ramnagar section showing stratigraphic position of the hominid teeth.

cates a Burdigalian affinity as well. In fact the approximate 18 m.y. datum for hominid existence in the Siwalik region is within expectations[9] and supports the earlier view of Andrews and Tobein[10] that the initial hominid diversification took place 'outside Africa and probably in central Asia'. The evolved character of the present *Sivapithecus* molar also indicates the pre-existence of its ancestral stock in Murree times as well and further suggests the Siwalik region to be the probable centre of hominid diversification and radiation. Further work, currently in progress in the area, is likely to substantiate the present find.

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Ultrastructural evaluation, an useful method for fish leucocyte identification

Clarias batrachus is an air-breathing fish inhabiting unhygienic hypoxic waters of the swamp. Because of its air-breathing habit and survival in hypoxic water it is used as one of the model species for aquaculture in swampy water. Routine differential blood count reflects the pathophysiological status of the fish in culture. Sometimes difficulty was encountered in differentiating various leucocyte types by light microscopy[1–3] and scanning electron microscopy[4,5]. Identification of leucocytes at ultrastructural level is more authentic[6] and therefore, forms the basis of the present report.

Live specimens of *C. batrachus* (62 g) were cold anaesthetized[7] and blood samples were obtained in heparinized micro-haematocrit tube from the caudal vein and centrifuged at 1000 *g* for 30 min to sediment the leucocytes. The plasma of the haematocrit tube was carefully removed and replaced by chilled (4°C) 1.5% phosphate buffered (pH 7.4) glutaraldehyde for preliminary fixation of the packed mass of the leucocytes. After 1 h, the fixative (1.5% phosphate-buffered glutaraldehyde) was removed and replaced by 1% phosphate-buffered (pH 7.4) osmium tetraoxide at 4°C for 2 h. After 2 h the buffy coat of the packed leucocyte mass was sufficiently hardened to be removed from the micro-haematocrit tube, dehydrated in ascending concentrations of ethanol and acetone, cleared in toluene and embedded in araldite. Ultrathin (60–90 nm) sections obtained through Reichert Jung Supernova Ultramicrotome were transferred to copper grids and stained in uranyl acetate and lead citrate and viewed under Philips EM-10 transmission electron microscope at SIC-EM facility, AIIMS, New Delhi.

A merz grid of known dimension was projected over the transmission electron micrographs to estimate the surface volume ratios of the various leucocytes.

Lymphocytes, thrombocytes and granulocytes were the three distinct types of leucocytes identified in the section. The lymphocytes are the smallest (cross sectional area – 4.79 μm^2) and the most abundant leucocytes discernible in the section (Figure 1 *a*). The large clefted nucleus (3.55 μm^2) occupies nearly 74% of the entire interior of the cell and is surrounded only by a thin (1.24 μm^2) rim of cytoplasm. The nucleus–cell (N/C) ratio was very high (0.74). The surface/volume ratios of each leucocyte and its nucleus were 0.865 and 1.2 respectively. The nuclear chromatin was abundantly distributed on the periphery and the central part of the nucleoplasm. These ultrastructural

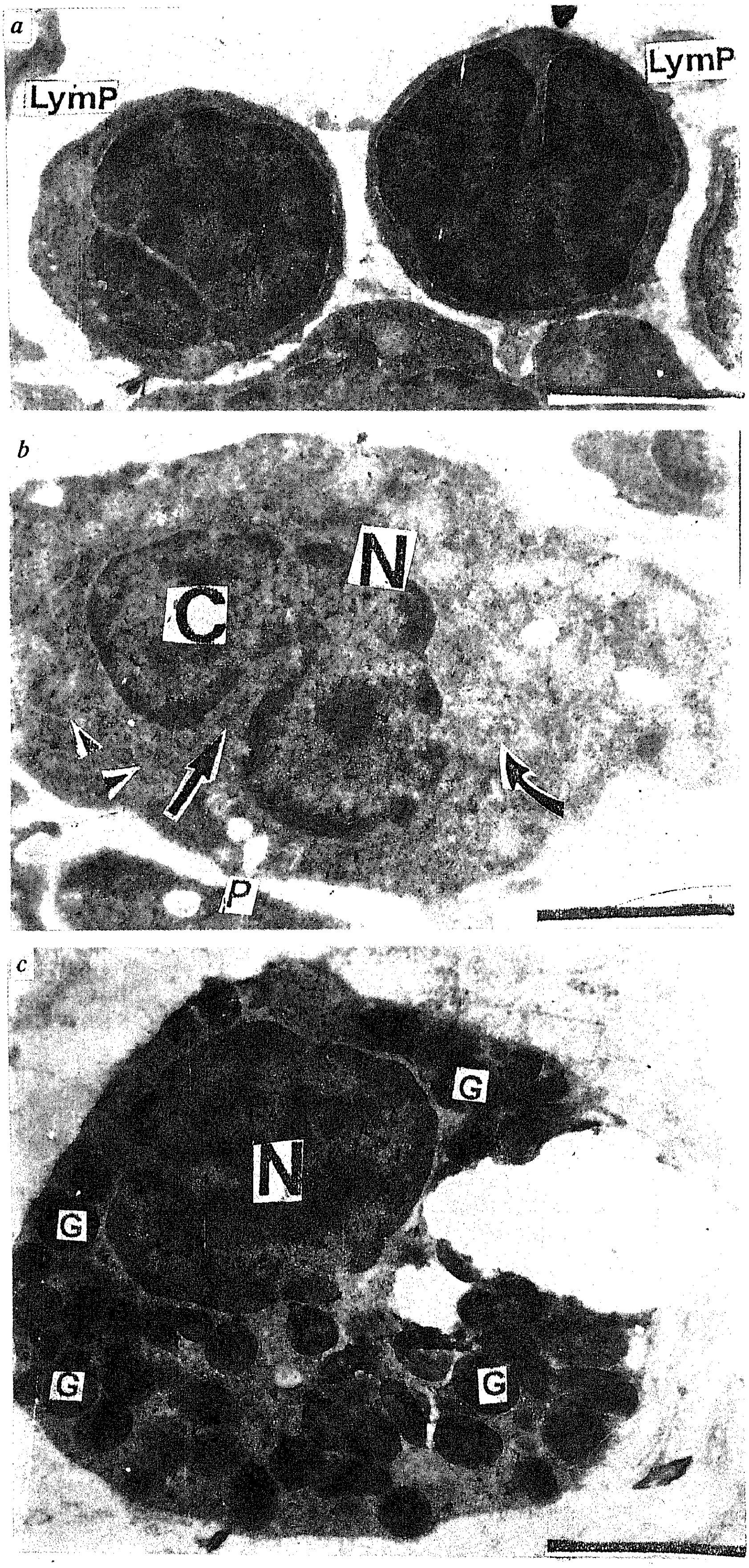

Figure 1 *a–c*. *a*, Transmission electron micrographs of the packed leucocyte showing lymphocytes (Lymp), bar = 1.33 µm. *b*, TEM of the fusiform thrombocyte showing chromatic (C), nuclear pore (arrow) of the nucleus (N). Pinocytic vesicles (P), electron dense material-filled vacuoles (curved arrow) and microtubules (arrowheads) are also seen, bar = 1.33 µm. *c*, TEM of granulocyte showing nucleus (N) and osmiophilic granules (G), bar = 1.33 µm.

details will be useful in identifying lymphocytes in the leucocyte sections of fish. The lymphocytes are important leucocytes with immunoglobulin (I_g) molecules on their surface membrane[8]. To the contrary of the high percentage of lymphocytes only a few thrombocytes were discernible in the leucocyte section of the air-breathing catfish. Each elongated fusiform thrombocyte was characterized by clefted nucleus (Figure 1 *b*) and its large size (18.07 μm^2). The nucleus (5.21 μm^2) occupies about 29% of the total cellular cross section with comparatively small (N/C) ratio (0.29). The surface/volume ratios of the cell and the nucleus were 0.50 and 0.93 respectively. Nuclear pores were clearly discernible in the nuclear membrane. The denser chromatin granules were mostly distributed on the periphery of the nucleus. However, centrally placed chromatin patches in the nucleoplasm were also seen. Presence of microtubules, clear vacuoles and vesicles full of osmiophilic granules were the other features of thrombocytes of the catfish in question. Each vesicle contains electron dense material similar to that of cytoplasm. Such vesicles loaded with secretory granules indicate their secretory nature. The granulocytes are larger (19 μm^2) with eccentrically placed nucleus (4.78 μm^2) and large number of osmiophilic granules of varied dimensions ($0.46 \pm 0.12\ \mu m^2$). The N/C ratio was comparatively smaller (0.25). Denser nuclear chromatin was more patchy in distribution. The cytoplasm was packed with numerous large, round to elongated osmiophilic granules (Figure 1 *c*). The surface/volume ratios of the cell and the nucleus were 0.42 and 0.88 respectively.

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Plant life under extreme environments

H. Y. Mohan Ram and Promila Gupta

There is no habitat in the world where some plant or the other will not grow provided there is some moisture and light[1]. It is the energy provided by these plants that sustains the life of all heterotrophic organisms. Plants cannot tolerate excessively high temperatures like some of the bacteria and fungi, but do occur under extremely hostile surroundings such as mountain tops, hot and cold deserts, water-logged conditions, coastal habitats, metalliferous soils and spoil heaps of old mines. Plant life of the arctic is highly specialized with an antiquity dating back to at least 2 million years[2]. The knowledge of the survival strategies should assist humans to learn the secrets of balance, productivity and sustainability and as references against which we compare areas which we have developed[3]. Even for our own survival and that of the future generations, understanding of the nature of the stress-tolerant genes and their introduction into agricultural, horticultural and forestry species either through conventional breeding or genetic engineering would be important.

A vast amount of literature exists on the adaptation of plants to extreme environmental conditions. This pictorial article essentially deals with natural history. Certain leads in ecology and physiology have been indicated; aspects dealing with pollution and biotic stresses are not covered.

Plant life in deserts

Deserts are hot or cold, often sand-covered lands receiving below 125 mm annual rainfall. Their soils are dry with abundant soluble minerals and scanty vegetation. Humans have learnt to overcome the perils of desert existence by finding sources of water, food and shelter. Deserts also provide some of the world's most awe-inspiring scenery with a spectrum of moods and quiet mystery. Roughly one seventh of the globe's surface is occupied by deserts.

Water is necessary and convenient for all life and is a molecule of common occurrence. Plants have an extensive root system which absorbs both water and minerals. Ditmer (cited in ref. 4) of the Iowa State University has made the astounding finding that the total length of roots (minus root hairs) formed by a single rye plant in a period of four months is 387 miles, covering a total surface area of 2554 sq. ft.! Plants lose large amount of water in transpiration through minute openings in the shoot called stomata, sometimes in excess of the quantity absorbed. If the stomata close down to check water loss, the plants would starve as they are the gateways for entry of carbon dioxide, the important raw material for photosynthesis. In evolutionary terms, transpiration is an unavoidable evil of plant life. Thus conservation of water and its usage is one of the greatest challenges faced by desert plants. Plants inhabiting deserts are termed xerophytes (xerophiles) and are further categorized by Levitt[5] as evaders, avoiders and tolerant plants. Plants occurring in hot deserts are slow-growing and may suffer mechanical or metabolic form of drought injury[6]. The latter is manifested in the form of decreased photosynthesis, protein metabolism and membrane permeability.

As availability of water is restricted in most deserts, at times only to a few days, evaders have to complete seed germination, establishment of plant, flowering and seed set in a brief span. After one or two showers, the parched soil turns green and the splendour of flowering is spread over the entire desert. The evaders are thus ephemerals that pass the rest of the year as seed. The avoiders have special structural modifications to reduce water loss. These include a thick outer layer (the epidermis), sunken stomata, waxy coating, rolling of leaves during drought, reduction in size or absence of leaves or their early detachment. In plants such as *Euphorbia caducifolia* of the Thar desert, *Fouquieria splendens* and *Opuntia* sp. of the Sonoran desert, the stem turns green to carry on photosynthesis.

Table 1 shows the enormous extent of root development in a few desert plants. In plants such as *Aqave salmiana*, the root to shoot ratio is low. In older perennials and shrubs of the Great Basin, Turkestan and some other deserts, it ranges from 1 to 9 (refs 7, 8).

A common morphological feature of desert plants is succulence of the above ground parts. Succulents occur in several distant and unrelated families such as Aizoaceae, Chenopodiaceae, Cactaceae, Crassulaceae, Euphorbiaceae, Asclepiadaceae, Liliaceae and Amaryllidaceae. This leads to the confusion between succulents and cacti and strictly speaking the latter belong to the Cactaceae. All the cacti are of New World origin,

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This article is based on a lecture delivered by H.Y.M. at Jodhpur on 2 November 1996 during the 62nd Annual Meeting of the Indian Academy of Sciences. We pay homage to all the plant scientists of the Central Arid Zone Research Institute, University of Jodhpur, notably Professor M. M Bhandari and the Botanical Survery of India Arid Zone Circle, Jodhpur, who have enriched our understanding of plant life in the Thar Desert.

Table 1. Extent of root penetration of a few representative plants growing in different deserts

Genus	Location	Root penetration (m)		Source
Alhagi	Asian desert	Depth	25	Daubenmire (1959)
Glycyrrhiza	Asian desert	Depth	15	Daubenmire (1959)
Andina	Brazalian desert	Depth	19	Daubenmire (1959)
Prosopis	Arizonian desert	Depth	20	Daubenmire (1959)
Tamarix	Saharan desert	Radius	40	Ladover (1928)
Larrea	Saharan desert	Radius	27	Ladover (1938)

although they have been introduced to the other continents where some have run wild. Succulents are able to absorb huge amounts of water during a heavy rain and store it for a long time. The saguaro cactus (*Carnegia gigantea*) of the Sonoran desert reaches a height of 15 m, lives for 150–200 years and weighs 9–10 tons[9]. Its shallow, wide-ranging roots and the surface of the fluted stem can gather as much as one ton of water after one or two bouts of rain. Additionally in some succulents, the stomata close during the day to prevent evaporation and open only during the night. Carbon dioxide is fixed in the dark in the form of carboxylic acids, which are converted into carbohydrates during the day. Such plants are called CAM (Crassulacean Acid Metabolism) plants.

The truly drought-tolerant plants are those which have the capacity to stand dehydration for prolonged periods without succumbing to it. Lichens, mosses, ferns and a few seed plants are included under this category. They are collectively called resurrection plants. A common example is *Selaginella* sp. sold as a curiosity at holy places in North India. The dried and curled-up plants turn green after a few hours of soaking in water. Among the most dramatic examples of resurrection plants are creosote bush – *Larrea divaricata, Carex physoides, Ramonda nathaliae, Myrothamnus flabellifolia, Chamaegigas intrepidus.* These stay glued to the soil almost appearing dead and dry, turning green and reviving even after one rain. They start respiring within 48-60 hours and begin to photosynthesize thereafter. The rose of Jericho (*Anastatica hierochuntica*) is unusual, as the dry plant rolls up and is blown around by wind. When it starts raining the plants recover, become stabilized and continue to grow.

The creosote bush produces chemicals that smell like coal tar in its shoot to dissuade browsing animals. Its roots also secrete certain substances (allelopathic chemicals) that inhibit the roots of other plants in the vicinity. The plant collects moisture from the dew or in case of an exceptional rain by sending out underground stems with mats of tiny roots to absorb every drop of water percolated into the soil. Another bizarre plant that has an amazing capacity to survive for over 1000 years in perennially arid conditions of the Namib desert is the gymnosperm *Welwitschia.* It is also endowed with the ability to collect dew from the air.

Sand-dunes are by far the most common habitats of the arid deserts. The dunes are formed by strong winds that carry the sand particles and pile them into mounds. Some dunes are stabilized, others partially stabilized or unstabilized and barren. Several herbaceous grasses, amaranths, legumes, cucurbits and asclepiads cover the sand by striking roots at nodes. The most common shrubs of the dunes in the Thar desert are *Calligonum polygonoides* (an excellent source of charcoal), *Commiphora wightii* (guggul), *Clerodendrum phlomidis, Haloxylon salicornicum, Lycium barbatum* and trees such as *Prosopis cineraria, Salvadora oleoides* and *Acacia senegal.* These provide food, feed, shelter or other materials for animals and people[9a].

Some herbaceous plants surviving cold stress in the frozen arctic region have flowers which show solar tracking; for example *Dryas octopetela, Papaver radicatum* and *Ranunculus glacialis.* The petals reflect the warmth of the sun's rays towards the centre of the flower to increase the temperatures to attract the pollinating insects[1]. The cushion habit plants such as dwarf azalea (*Loiseleuria procumbens*) and dwarf campion (*Silene acaulis*) in the cold deserts increase the inner temperature by 15°C above the ambient temperature[10] and also humidity. These plants have the unusual capability of making a net carbon gain for the entire year in a brief growing season, complete development and reproductive cycle with minimal thermal input[1]. As they cannot invest sufficient material for sexual reproduction, they turn to vegetative propagation[11]. Shivaji and Ray[11a] have reviewed the survival strategies of microorganisms adapted to extreme cold conditions prevailing in Antarctica.

Salinity

Very often the aridity caused by low rainfall and extremely high or low temperatures are compounded by high salinity in the soil, resulting in severe problems in absorption of water and curtailing productivity. Plants that survive high salinity are called halophytes. Among these, members of family Chenopodiaceae, especially the species of *Atriplex* are notorious. Collectively called the saltbush, the species of *Atriplex* show accumulation of ions against concentration gradient. Plants such as *Suaeda, Salsola, Kochia, Haloxylon, Portulaca, Cressa, Sesuvium* and *Tamarix* commonly occur in the Thar desert. Some species have the ability to store and secrete salt through special glands on the leaf surface. Additionally, in the ice-plant *Mesembryanthemum crystallinum,* a native of the Namib desert, the presence of salt secreted on the surface enables absorption of moisture from the humid air.

Several useful species of *Atriplex* have been introduced to India for cultivation on saline soils for meeting fodder and fuel requirements and also for stabilizing mineral soils. As a result of heavy irrigation and poor drainage, many parts in India have been experiencing increased salinity. It is common to see large patches of white crust of salt on the surface of the clayey soils containing sodium chloride and other salts in Haryana, Punjab, Rajasthan and Western UP. Many crops are sensitive to salt whereas a few are moderately tolerant. The distribution of wild relatives of cultivated plants in the saline soils as well as varieties of rice that are salt tolerant and the mechanism of salt tolerance have lately become important aspects of agricultural research. An increase in root to shoot ratio has also been reported in response to salt stress[12,13].

Mangroves and plants of salt marshes

Plants that inhabit coastal regions and estuaries and have become adapted to survive under inundated salt water conditions constitute the mangrove vegetation. Some are viviparous and shed their germinated fruits in tidal waters to facilitate their establishment. Physiologically mangroves not only face the problem of absorption of water from highly saline conditions but suffer from insufficiency of oxygen in their roots. The angiospermous mangroves and swamp cypress (*Taxodium distichum*) produce characteristic vertical roots which grow in a negatively geotropic manner above the water level and serve the purpose of aeration (pneumatophores). Mangroves are ecologically important as they prevent coastal erosion. There has been a severe destruction of mangroves, on account of the valuable firewood and tannins they yield.

Coconut is among the most useful plants which is highly salt-tolerant. *Casuarina equisetifolia* and other species of *Casuarina* were introduced to India from Australia primarily for checking coastal erosion. They are presently grown even inland as fast-growing trees, capable of fixing atmospheric nitrogen with the association of *Frankia.* As the wood can be burnt even when still green, it is used as an important source of firewood. Herbaceous plants with excellent coastal sand-binding properties include *Ipomoea aquatica* and *Spinifex squarrosa.* The latter is being recommended for preventing coastal erosion in the Lakshadweep islands.

Aquatic plants

Water is essential for plant life but its over-abundance leads to several problems. This is particularly true of those plants in which photosynthetically active parts are submerged in water or float on the surface permanently or at least for several months each year[14]. About 2% of all the flowering plants known in the world live in water and more than 50% of these are represented in the Indian subcontinent[15]. Evolutionarily plants migrated from water to land. However, when the reverse process occurred, plants had to undergo a wide range of structural, physiological and biochemical changes. Notable modifications of aquatic plants include a poor root system, high reduction in mechanical tissues, development of large air spaces which interconnect the entire plant and a high propensity for vegetative propagation. Plants living in water and those subjected to flooding show different degrees of oxygen shortage[16]. Aquatic plants with floating leaves such as *Nymphaea* and the Indian lotus (*Nelumbo nucifera*) have rhizomes and roots buried in the anoxic sediments of shallow freshwater lakes. As oxygen supply by gaseous diffusion to the submerged parts is difficult, these plants have developed a pressurized diffusion system, also termed thermal transpiration (thermal osmosis). Addressing the French Academy in 1841, Raffeneau-Delile of the Faculty of Medicine at Montpellier, reported his curious observation of the escape of gases from partially submerged lotus leaves in Egypt. Since then, several other scientists have pursued the study of gas-flow through the air-space system in emergent water plants.

John Dacey, a doctoral student working at the Michigan State University on the flow-through ventilation system in the yellow water lily (*Nuphar advena* Ait = *N. lutea*) has been largely responsible for resurgence of interest in this study. Using sensitive GC analysis and by injecting ethane into the stalks of young leaves, Dacey[17] was able to demonstrate the downward movement of the gas into the rhizome and its escape through stomata in the older leaves. He noted that the heat absorbed by the young leaves (influx leaves) converts water into vapour, which is pushed downwards under pressure along with other gases through the intercellular spaces to their rhizome and their exit through the petioles and leaf blade of older leaves (efflux leaves) on the same rhizome branch (Figure 1). This process enables supply of oxygen to the roots, rhizomes and rhizosphere and the movement of respiratory carbon dioxide to the leaves for photosynthesis[17-19]. Dacey and Klug[20] also showed that these waterlily plants transferred methane gas formed by anaerobic methanogenic bacteria from lake sediments to the atmosphere. This mechanism has been shown to operate in other submerged plants for the supply of oxygen to parts below water[21,22] and has been aptly called the 'biological steam-engine'. In a recent review, Singh and Singh[23] have reviewed the role of plants such as *Typha* and rice in methane emission from the wetlands in India.

The Podostemaceae – A strange family of aquatic plants

A good deal of information is available on the stresses experienced by submerged, free-floating and emergent but rooted aquatic plants. There are gaps in our knowledge about a highly interesting family of flowering plants, the Podostemaceae. They occur almost exclusively under cataracts and fast-flowing rivers, submerged but firmly attached to rocks by means of rhizoids that secrete a gummy substance[24,25]. They have a thallus-like plant body that has no air spaces, unlike other water plants. Members of the sub-family Podostemoideae lack differentiation into root, stem and leaves and can be easily mistaken for algae, lichens or liverworts. Generally so long as the plants lie 20–25 cm below water, they never enter the flowering phase. However, a drop in water level causes initiation of flower buds. Flower opening, fruit and seed development require exposure to air. Thus sexual reproduction in these plants appears to be regulated by water stress. These aspects have been substantiated by experimentally grown plants in our laboratory.

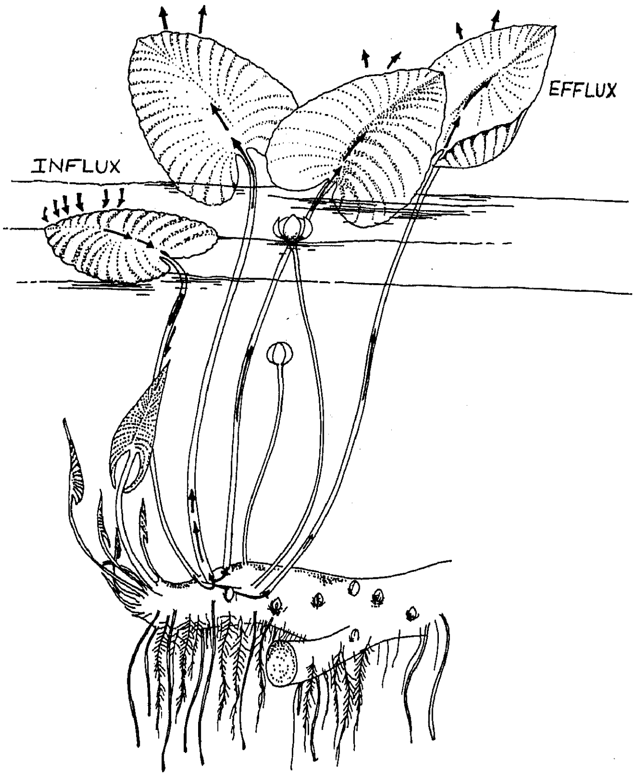


Figure 1. Ventilation in *Nuphar* advena, showing the path of the gas through flow (Drawn after Dacey).

Water logging and flooding

Most trees are sensitive to water logging. However, there are many exceptions such as the massive trees of the Amazonian forests adapted to tolerate flooding for several months. The mechanisms that support life under anaerobic conditions of these plants are being studied[16].

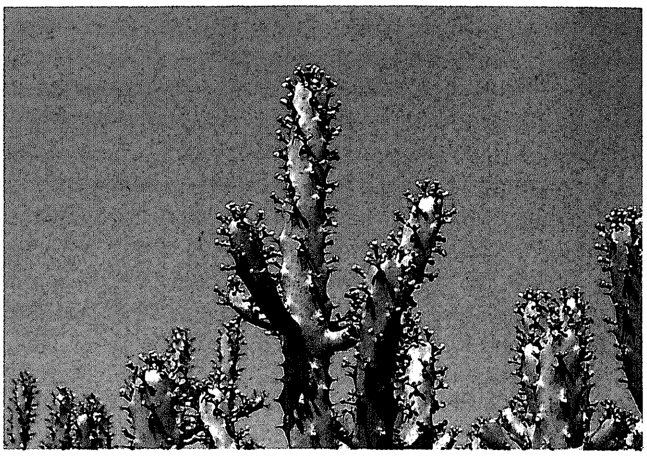
Certain deepwater rices are known to respond to flooding in late monsoon. They branch at the higher nodes, show rapid internodal elongation when submerged due to rise in the level of water and produce adventitious roots at the upper nodes. Submergence stimulates the synthesis of ethylene gas which helps in cell lengthening response to the hormone gibberellic acid[26]. Both cell division and cell elongation are responsible for the rapid stretching of rice plants (often 25 cm in one day) under flooded conditions[27]

Metallophytes

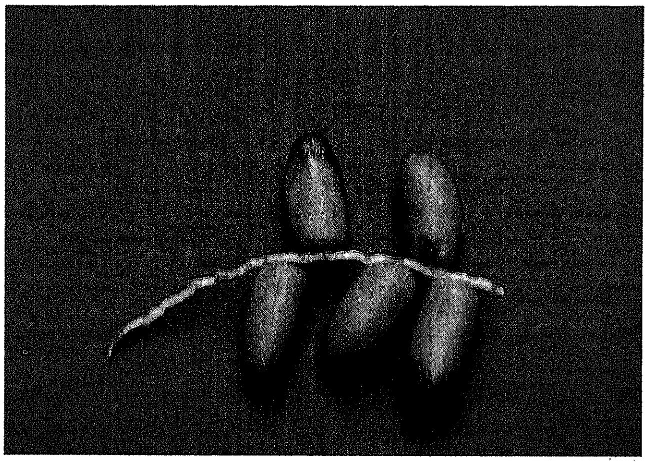
Certain elements are essential for all organisms; some are needed in relatively large amounts (macro or major elements) and others in small amounts (micro elements). Unlike animals, the majority of plants do not need sodium and iodine. Curiously plants accumulate a wide spectrum of non-essential elements at concentrations several hundred-fold higher than that in the soil or water. Barring drinking water and pollutants, all the essential minerals needed by animals and humans are derived directly or indirectly from plants. Ecologists have studied the types of vegetation occurring on metalliferous soils, mine-tailings and abandoned mines and have identified plant communities and populations of individual species growing on them. It is possible to associate indicator species that reflect the concentration of a metal in the soil. This interesting branch of science termed geobotany, has been helpful in prospecting for minerals and also in bioremediation and recovery of contaminated soils to productive lands.

Baker and Walker[28] have classified plants that grow on metalliferous soils into three groups: (i) Metal-excluders – those that accumulate large amounts of metal(s) in their roots but prevent their translocation to the shoot. (ii) Indicators – which contain metals both in root and shoot but reflect the levels of metal present in the soil. (iii) Hyperaccumulators – species which con-

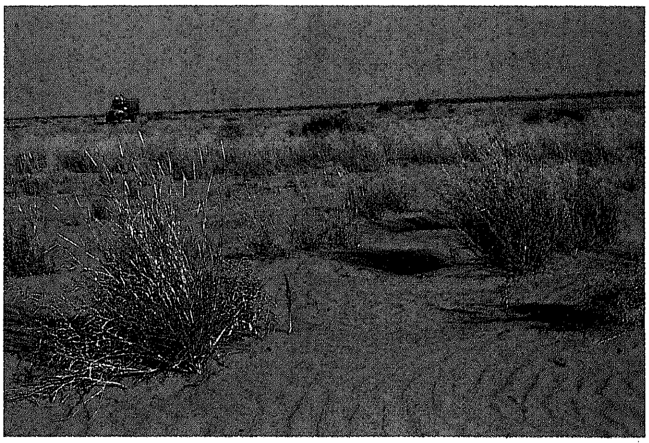
A close-up of the female inflorescence of the date palm *Phoenix dactylifera*. The palm is hardly and grows near oasis in the Central Asian deserts with 'feet in water and head in fire'.



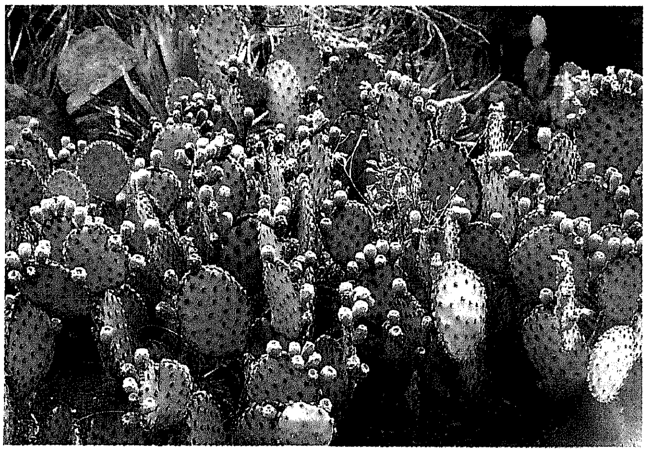
A close-up of *Euphorbia caducifolia* of the Thar desert. The green stem is photosynthetic and contains a milky latex. Many succulents are erroneously called cacti. (H. Y. Mohan Ram).



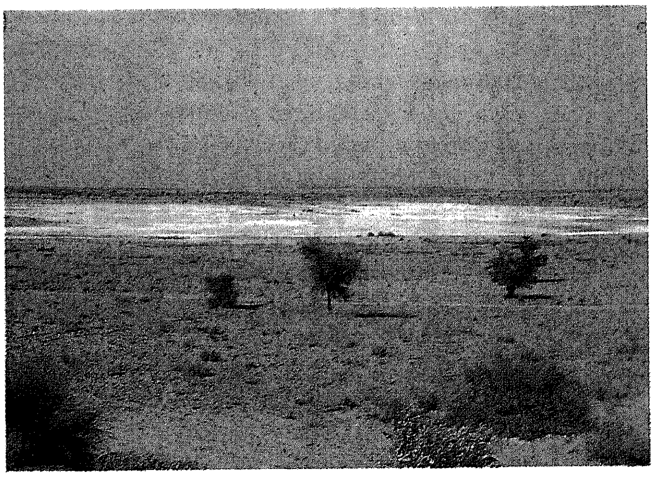
Mature fruits of date, eaten fresh or dried. Before the oil boom, date was an important item of trade in Middle East. (H. Y. Mohan Ram).



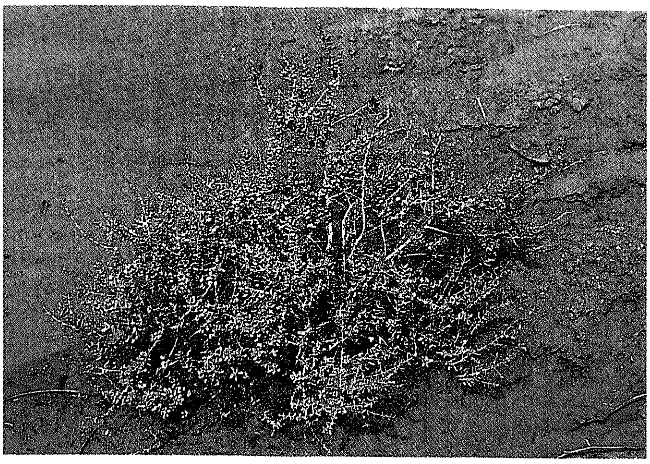
Ferrocactus wislizerii, the fish hook barrel cactus from Arizona USA, with orange flowers. Browsing animals are kept away. (H. Y. Mohan Ram).



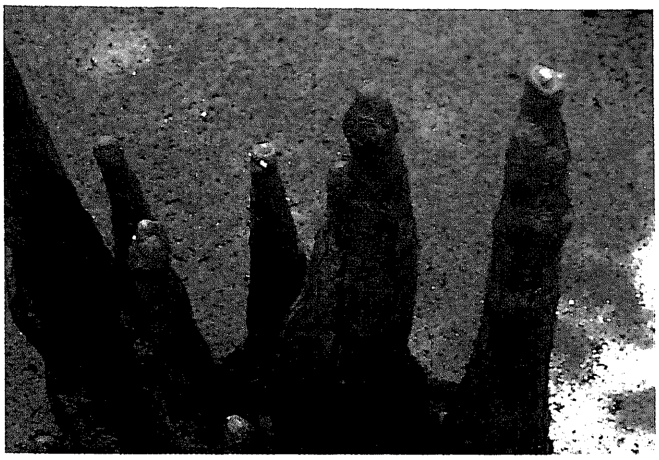
Opuntia or prickly pear of the Sonoran desert in N. America (this particular species has no barbed bristles). A leafless succulent, it stores water in the tissues and can tolerate 60°C for a few hours. (H. Y. Mohan Ram).



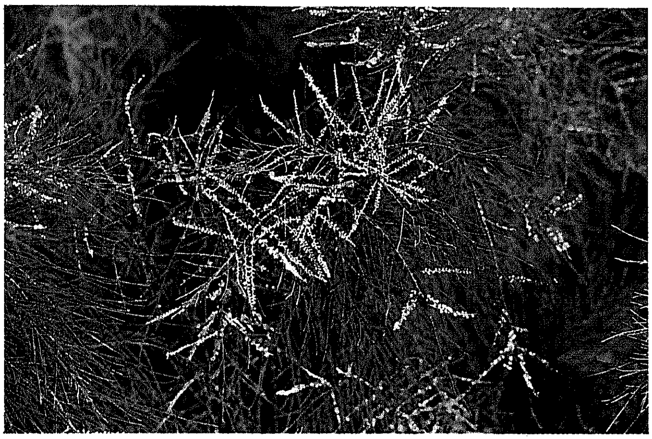
Suratgarh, Rajasthan. Salt deposition on the soil caused by rise in water table due to irrigation. (M. M. Bhandari).



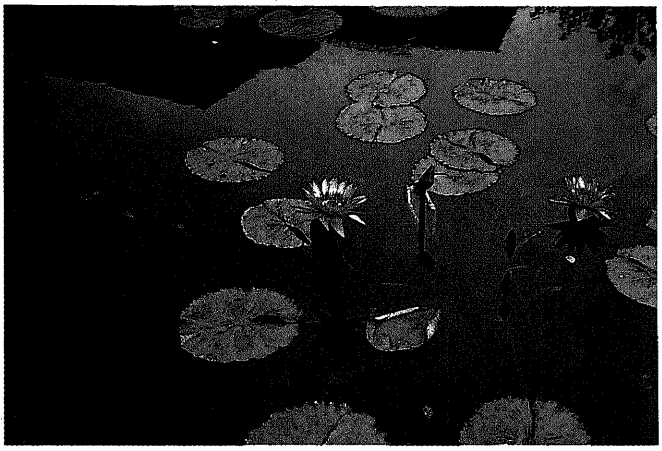
Suade fructicosa a greyish-green, herbaceous halophyte of the Thar desert, it has an extensive root system. (M. M. Bhandari).



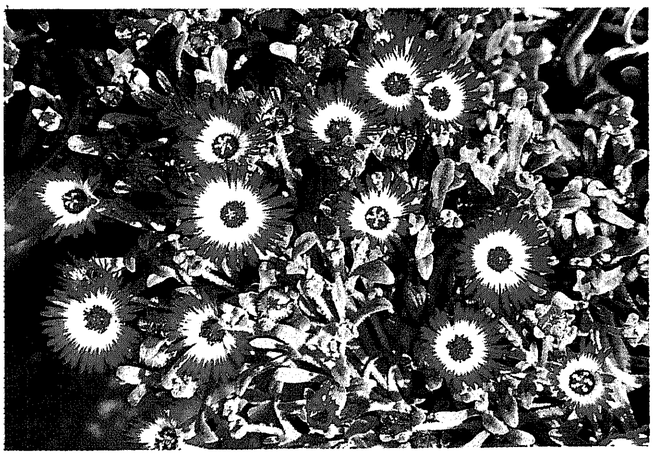
Pneumatophores of swamp cypress. Some of the roots grow vertically above water and assist in gaseous exchange. (H. Y. Mohan Ram).



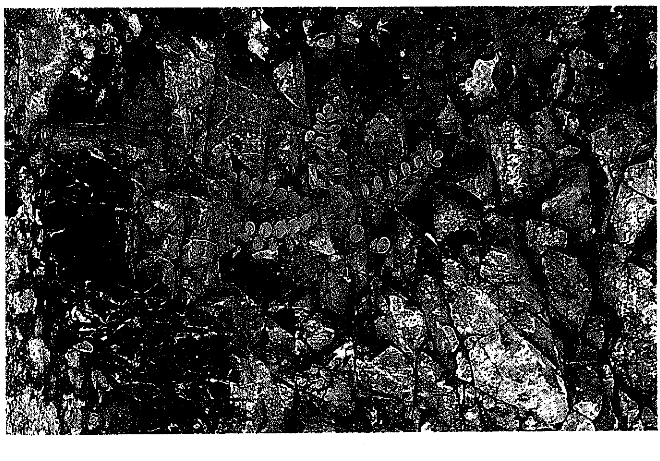
Tamarix sp. A typical plant of the salt rich soils of Thar desert, it has an extensive root system. (M. M. Bhandari).



Nymphaea, a rooted aquatic plant with floating leaves and beautiful flowers has a 'steam engine' mechanism for aerating the buried rhizomes. (H. Y. Mohan Ram).

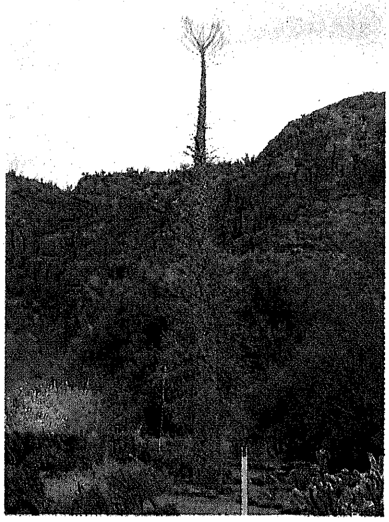


The ice plant, a native of the Namib desert in South Africa is now grown throughout the world as an ornamental. The surface of the plant has vesicles looking like ice crystals. The secreted salt helps in the absorption of moisture in the air. (Rajesh Tandon).

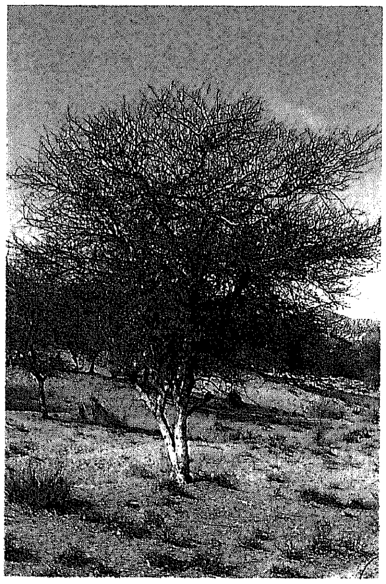


Astragalus graveolens, a leguminous weed established on a limestone quarry by scientists of the University of Delhi. Seeds embedded in sodium alginate along with the symboitic nitrogen fixing bacterium *Rhizobium* are used for reclamation. (Anupam Joshi).

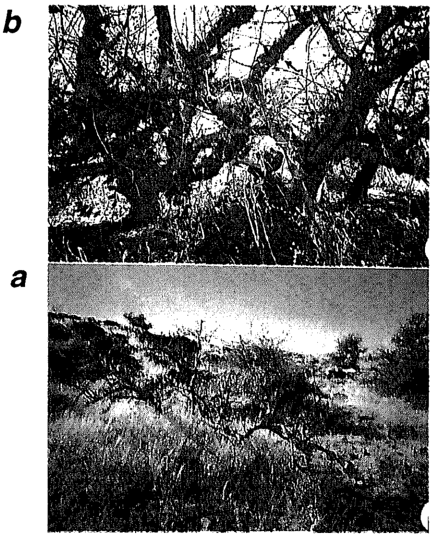
Spinifex squarrosa, a trailing grass, effective as a sand binder along the southern sea coasts of India. It is popularly called Ravana's whiskers on account of the stiff, sharp leaves. (Pratibha Pande).



The boojam tree (*Idira columnaris*) of Arizona, USA. It gives the impression of being upside down. (H. Y. Mohan Ram).



Kurrta. *Acacia senegal* is the source of gum arabic. The tree yields fodder, edible seeds and firewood. (Rajesh Tandon).



a. A rocky slope with thorny vegetation near Jodhpur. In the foreground is seen the innocuous, leafless shrub of guggul, amidst dry grass. ***b.*** A portion enlarged to show the tangled mass of branches of the shrub. The yellowish resin produced by incising the stem dries up into a brown mass. Guggul is an ancient Indian drug and its hypolipidaemic, hypocholestrolemic and antiarthritic effects have been reestablished by Indian scientists. 'Guglip' is presently sold in the market. The plant is threatened by over-exploitation.

centrate metals in the above ground parts, far in excess of that is present in the soil. Among the hyperaccumulators, those that take up heavy metals from soil are of tremendous importance. They have attracted attention for understanding the ecophysiological mechanisms and importantly the genetic basis of their endowment[29].

On a dry weight basis, hyperaccumulators may contain 0.1% of Ni, Co, Cu and Cr and Pb or 1% of Zn in their leaves. Tiagi and Aery[29a], working at the Udaipur University have studied the zinc deposits at the Zawar mines. They have noted that the balsam plant (*Impatiens balsamina*) and *Triumfetta pentandra* constitute an important vegetational unit. In the Rajpura–Dariba zinc bearing belt, *Kickxia ramosissima* and *Bidens biternata* show the highest constancy and fidelity on these soils.

The list of hyperaccumulating plants is growing steadily. Among these, species of the Brassicaceae (Cruciferae or the mustard family) are predominant in the temperate regions whereas the Euphorbiaceae represent their counterpart in the tropics[30]. Baker and coworkers[31] have reported that the Caledonian tree *Sebertia acuminata* has more than 11% of Ni in its latex on a dry-weight basis.

When hyperaccumulators enter the food chain through browsing wild animals or domestic livestock or poultry, there are chances of reaching acute toxicity levels in them as well as in humans. The classical examples of this condition are selenium-accumulating species of *Atriplex* and *Haplopappus*. Selenium replaces sulphur in the amino acids cysteine and methionine, resulting in various disorders in humans and livestock. 'Blind staggers' is a condition caused by excess selenium in livestock, consuming 100–1000 ppm in the forage. It results in impairment of vision, weakness of limbs and respiratory failure. Cattle and horses feeding on plants containing 25 ppm of selenium, suffer from 'alkali disease' with symptoms such as lack of vitality, loss of hair, sterility, atrophy of hooves, lameness and anaemia[32].

The copper flower (*Haumaniastrum katangense*), a common weed in the world's richest Zaire copper and cobalt belt in Central Africa, is a classical example which contains more than 0.1% of Co or Cu in the dried leaf-tissue.

Several scientists including botanists have a prejudiced view of the utility of a herbarium. Besides serving as a systematically arranged repository of dried plants, (authentic representatives of described and named species) a herbarium serves many other important functions. Recently pieces of leaves removed from herbarium specimens have indicated the amounts of heavy metals accumulated by plants 10–200 years ago from specific sites, thanks to sensitive methods of analysis. It is possible to judge the fluctuating levels of certain metals as well as levels of pollution over any given period of time by comparing the values in live plants with those in preserved plants of the past.

Mechanisms

The enormous biodiversity that exists in plants underlines their ability to adapt themselves to a wide spectrum of environmental conditions, as narrated above. Whether it is drought, cold, salt or metal stress or a combination of some or all of these, the end result is a dehydration stress. As early as 1958, Levitt[33] speculated that plants may have a general mechanism of resistance to every stress. At the morphological level wilting, leaf rolling and decrease in stomatal aperture, succulence, leaflessness, etc. constitute a general mechanism for conserving water[34]. At the physiological level, reduction in evapotranspiration and decrease in water potential are major manifestations.

How do plants sense stress signals?

There has been a revival in the study of signal communication in plants[35]. For example, how do roots perceive water stress and transmit the signal to the shoot to cause stomatal closure? The chemical communication with stress hormone abscisic acid (ABA) requires transcription and translation and hence is a slow process requiring at least two hours. The electrical signal (action potential) is rapid and acts in seconds. However, conduction of electrical signals has some limitations where long distances are concerned[35]. We are also in the dark regarding signals which transmit other stresses such as cold, salinity or high metal concentrations.

Stress- induced chemicals

Biochemical studies have revealed similarities in processes induced by various abiological stresses, leading to accumulation in plant tissues of compounds such as ascorbate, glutathione, α-tocopherol, betaine, proline and other amino acids, quaternary ammonium compounds, polyamines, sucrose, polyols (mannitol, sorbitol, pinitol) and oligosaccharides[36]. Additionally changes in the activity of certain key enzymes, gene expression and biosynthesis of ABA have also been noted.

Plants have developed mechanisms to prevent toxic effects of heavy metals on their metabolism. Many plants compartmentalize ions by storing them in their vacuoles. Inside the vacuoles, they may be chelated by citric or malic acid and rendered inactive. Recently certain polypeptides with the general formula γ(Glu–cys)*n*-Gly (where n = 2–11) have been identified in plants. These compounds termed phytochelatins or metallochelatins are believed to be induced by a specific metal. There is some evidence that as in animals, in plants also, there are genes that code for metallothionein-like proteins[37] but these are synthesized mainly in response to heavy metals[38–40].

Role of heat shock proteins in thermotolerance

Unlike certain archaea (such as *Methanococcus jannaschii*) and fungi which have the unusual propensity to survive and function normally under temperatures in the range of 80–100°C, most flowering plants are unable to stand temperatures above 50°C. The prickly pear (*Opuntia* sp.) occurring in the deserts of North America is shown to tolerate 60°C for a few hours. The role of heat shock proteins (HSPs) in thermotolerance of crop plants has been recently reviewed by Viswanathan and Khanna-Chopra[41]. These authors have also discussed the various heat-avoidance mechanisms which are significant in production of crops, specially those grown in the tropical and sub-tropical countries. That heat shock proteins are induced as a result of newly transcribed mRNA is universally accepted in a wide range of organisms[41–45]. This unique response was first reported in the fruit fly *Drosophila melanogaster*, where the heat-induced chromosome puffing was followed by a high-level expression of HSPs[42]. The synthesis of these proteins leads to acquired thermotolerance.

There is increasing interest in the identification of genes and their products that regulate tolerance to physical stresses in plants. This topic was discussed in depth at a workshop entitled 'Physical stresses in plants' held in Maratea, Italy[46].

Dehydrins

During the long course of evolution, several groups of plants have attempted to develop the seed habit but successful seed plants have been recorded from the upper Devonian, approximately 345 million years ago[47]. There is no structure equivalent to a seed in the entire animal world. Generally, seeds result from sexual reproduction in gymnosperms and flowering plants, capable of developing into a new individual. A seed that contains an embryo, stored food and protective coverings represents suspended animation as it is neither the beginning nor the end of plant growth. Unlike the vertebrate embryo which upon maturity develops into a young individual, in a seed all growth ceases for a brief or a prolonged period of time. A unique feature of most seeds is that they show a high degree of desiccation even though the surrounding parts may have abundant moisture as in watermelon, tomato or lotus. Seeds are known for their longevity and viability even under extremely severe environmental conditions. The most well-studied is the lotus seed, estimated to retain its germinability even after 1000 years[48]. Seeds of the arctic lupin (*Lupinus arcticus*) collected from a hemming burrough in the Yukon province of Alaska are estimated to retain viability even after 10,000 years, although some scientists are doubtful about this claim[48].

Understandably there has been a tremendous amount of interest in finding the mechanism by which mature embryos are protected from excessive dehydration to survive long periods of time and come back to life and establish plants when conditions become favourable for germination. In this context the discovery of dehydrins has been of special significance. The term dehydrin is synonymous with 'Late Embryogenesis Abundant' proteins designated as belonging to the LEA D-11 family. These were initially identified in investigations made on proteins and mRNA populations during the maturity of cotyledons of the cotton plant and subsequently in barley, rice, oil seed rape and wheat[49,50]. In mature embryos, they may reach levels in excess of 1% of total soluble proteins[51]. The LEA proteins can also be induced by ABA in zygotic as well as somatic embryos[52]. According to Close[34] dehydrins are the most conspicuous of soluble proteins which are formed in response to drought, cold, salinity stresses and also during normal seed maturation. They range in size from 9 kDa to 200 kDa without showing resemblance to any known enzyme sequence[53,54].

Xu *et al.*[55] have studied the expression of a LEA protein gene *HVA 1* from barley (*Hordeum vulgare*) and have observed that it confers tolerance to water deficit and salt stress in transgenic rice when this gene is introduced. Some LEA proteins when present alone are not sufficient to confer desiccation tolerance but require the presence of other solutes like sugars to be effective[56–58].

Phytoremediation

Phytoremediation is a new technology employed for removing excessive toxic metals and pollutants from soils or contaminated aqueous medium. Kaizer Jamil[59] has shown that water hyacinth can accumulate large amounts of Hg, Cd, Zn, Pb and Cr from solution containing salts of these elements. Ozimek[60] has demonstrated the ability of several aquatic plants to cycle heavy metals from sewage waste. Both algae and large flowering water plants such as water hyacinth and *Phragmites* are being used for removal of heavy metals from contaminated waters. It is believed that bioremediation with the use of specific plants is a relatively inexpensive operation. It is also possible to recover heavy metals by incineration.

Heavy industrialization, uncontrolled discharge of factory effluents from smelting, electroplating and leather industries, emission of organic lead in the atmosphere from automobile exhaust are causing accumulation of dangerous amounts of metals in our environment. The possibilities of a cleaner and greener planet by using plants and employing methods which are simple, safe and dependable, are keenly awaited.

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Bacterial ice nucleation: Prospects and perspectives

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In this new emerging era of biotechnology, ice nucleating proteins find varied applications in different fields like artificial snow making, freeze texturization of foods, freeze drying, freeze concentration, detection of bacterial pathogens and also in the control of frost damage to plants. However, the commercial exploitation of this novel biobased and energy saving technology is still under consideration.

BIOLOGICAL systems to initiate a physical process are not new to man. Perhaps the best characterized biological indicators are bacterial ice nuclei, which trigger crystallization of ice in super-cooled water. Detailed characterization of ice nuclei has been carried out in certain bacteria of plant epiphytic communities like *Pseudomonas syringae, P. fluorescens, P. viridiflava, P. coronafasciens, Erwinia herbicola, E. ananas, E. uredovora, E. corotovora, Xanthomonas campestris* and *X. translucens*. In all the strains investigated so far, the phenotype is encoded by a single gene[1–5]. The product of this gene expression is a membrane-bound protein. This protein, alone or in combination with membrane phospholipids, mimics the structure of an ice nucleus and thus, acts as a template for ice formation which is responsible for imparting ice nucleating activity to the bacterium. Such bacteria are referred to as ice-plus (ice$^+$) or ice nucleation active (INA) bacteria.

Ice used in daily life is created either due to ice nucleators or due to secondary nucleation. Ice and water can co-exist in equilibrium at 0°C provided heat is neither added nor removed. Removal of heat causes water to convert to ice without need of nucleation. The system becomes totally solid, maintaining a temperature of 0°C. But, when ice is initially absent in the system, solidification follows a different path. When primary nucleation proceeds to crystal growth upon cooling, the temperature of the system falls below freezing point, called super cooling, before nucleation. Nucleation is a necessary step in order to surmount an energy barrier (activation energy) before water converts to ice. Thus, nucleation can be defined as a process that generates within the supercooled state of water (metastable mother phase), the initial fragment of a more stable phases. These fragments then spontaneously develop into gross fragments and the phase change is complete.

Types of ice nucleation

Nucleation is of two different types – homogeneous nucleation (that occurs in 100% pure water) and heterogeneous nucleation (that occurs in impure water). Heterogeneous ice nucleation is common in nature, where water aggregates in a crystalline arrangement and suspended foreign bodies, surface film or wall of the container serve as nucleating agent[6]. A decreased degree of super cooling is required for the heterogeneous ice nucleation. Many organic and inorganic substances like silver iodide[7], phloroglucinol dihydrate, alpha phenazine, metal aldehyde, kaolinite, covellite, magnetite[8], cholesterol[9], amino acids[10], lectins and proteins[11] are well known ice nucleators. However, biological ice nucleators are also well established. Among all these, INA bacteria are the most active.

Most of the INA bacteria are inhabitants of plant surfaces and some of them are also plant pathogens[12]. In general, they are gram-negative nonspore forming rod-shaped bacteria. Some of them are known to produce plant toxins (syringomycin from *P. syringae*) but are non-toxic to humans. These organisms have faster growth rate[13] and spread through aerosols[14].

Molecular biology of ice nucleation

INA protein, conferring ice-nucleating property to a bacterium is encoded by a single gene located on a small stretch of chromosomal DNA whose size ranges from 3.4 kb to 7.5 kb. The *Ina* genes that have been sequenced completely are from genus *Erwinia* (*InaA, InaU, IceE*), *Pseudomonas* (*InaZ, InaW*) and *Xanthomonas* (*InaX*). The *Ina* gene contains one open reading frame (ORF) of ~3600 bp, of which 80% consists of a series of imperfectly repeated DNA sequence with lengths of 24, 48, 144 bp. The DNA sequences of the ORF are highly homologous, whereas the sequences lying outside the ORF are usually non-homologous and

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the translation product is comparatively more conserved than the DNA sequence[15]. INA proteins produced by different INA$^+$ bacteria, immunologically cross react with each other indicating their similar antigenic structure. The translation product of INA gene is predicted to contain 1200 amino acids and has a molecular weight of around 120 kDa, comprising of contiguous repeats of a consensus octapeptide (Ala–Gly–Tyr–Gly–Ser–Thr–Leu–Thr) (ref. 1).

INA protein consists of three distinguishable domain structures – a unique N-terminal domain (~15% of total sequence), which is relatively hydrophobic and contains the membrane anchor for ice nucleation protein; a unique C-terminal domain (~4%) which is rich in basic residues and very hydrophilic; and a central repeating domain (81%), which is hydrophilic and rich in alanine, glycine, tyrosine, threonine, serine and leucine. This central domain can be sub-divided into three regions. The first two of these repeats consist of 48 peptide units of high fidelity; each 48-peptide unit can be further divided into three 16-peptide repeat units of medium fidelity; again, each 16-peptide unit can be further divided into two 8-peptide repeat units of low fidelity in which only two of the eight peptides are strongly conserved. The third region of the repeating domain contains only an 8-peptide repeat sequence (Figure 1).

The repeating domain is naturally suited to formation of a repeating template structure. This domain is rich in amino acid residues with neutral hydrophilic side chains capable of forming hydrogen bonds with water molecules[2,16]. Removal of several 8-amino acid repeating blocks lowers the nucleation-threshold temperature but does not abolish the nucleation activity[17]. The symmetry of this sequence is similar to that of certain crystal planes of ice[2,18]. Highly active bacteria contain only a few thousand copies of protein in each cell[19–21]. The repeating domains of this INA surface protein are capable of organizing water into an ice-like structure in three dimensions. The secondary structure of the protein is thought to be a beta-pleated sheet, punctuated by 5 to 6 turns per 48 amino acid sequences[22].

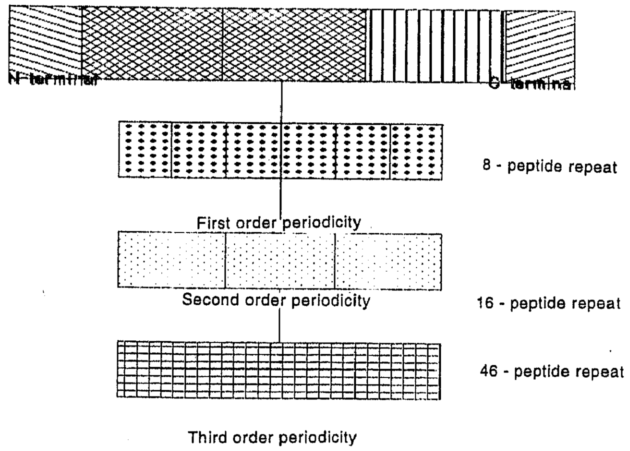

Figure 1. Diagrammatic representation of INA protein. (Adapted from ref. 52).

Factors affecting ice nucleation

Several chemical compounds known to disrupt membranes have been shown to decrease or abolish bacterial ice nucleation activity. Loss of membrane lipids causes loss of ice nucleation activity[23,24]. Treatment of bacterial cell wall with lipases, lipid extracting agents, quaternary ammonium compounds, detergents and dyes, etc., decreases the ice nucleation activity. The treatment of bacterial cell membrane with proteases and protein denaturants[25] affects functionality of INA protein. The change in temperature also causes disaggregation of INA protein[21], antibodies[26] and antifreeze glycopeptides[27], which physically blocks the water-binding surface of INA protein. Nucleation threshold temperature and nucleation frequency of INA$^+$ bacteria depend upon the dynamic assembly of multiple, membrane-bound aggregates of ice-nucleation protein. Though in most cases, INA protein is observed in cell membrane, *E. herbicola*, on the other hand, shed ice nuclei into medium. These are released as membrane vesicles of size 50–200 nm in diameter, which are active at –2 to –10°C and show similar freezing spectrum like that of intact nuclei[28]. These vesicles can also be stored at 0 to 4°C for weeks with no loss of activity. But, heating of these cell-free nuclei at 30°C causes rapid and irreversible loss of ice-nucleation activity.

Nucleation capability mainly depends upon the cell concentration. If the cell number/population is high, the temperature required for nucleation will also be higher. The dead cells also retain INA$^+$ activity, if there is no lysis. Approximately, 10^6 cells are required at –2°C to initiate nucleation, but at very high cell concentration (> 10^7 cells/ml), nucleation activity is independent of cell number/population. The other factors that contributed to the nucleation efficiency of INA protein are its lattice match and symmetry, water solubility, size of nucleation site, polarization ability, hydrophobicity, surface charge, number and strength of surface sites capable of absorbing water molecules[29].

Assay for ice nucleation activity

The best and rapid method for measurement of bacterial ice nucleation activity is droplet freezing assay. In this process, INA$^+$ bacteria are diluted and about 10 µl sample mix is placed on hydrophobic surface which remains floating on the surface of a refrigerated bath. The temperature is lowered step by step and at each point, the number of droplets frozen is counted to estimate the nucleation efficiency[30,31]. The common factors which probably affect bacterial ice nucleation assay are temperature, pH and volume of the drop, heat transfer capacity of cooling surface, concentration of cells, the ratio of ice nuclei per cell, time period and the temperature at which droplets are held.

Contrary to INA proteins, antifreeze glycopeptides seen in some fish, spiders, mites, plants, fungi (*Pleurotus ostreatus, Flamulina velupites*), bacteria (*Micrococcus cryophilus, Rhodococcus erythropolis, P. putida*)[32] interact directly with ice crystals and stop the increase in size of ice crystals. As it binds to surface of ice crystal embryo, it physically blocks part of the water-binding surface of INA protein. This results in neutralization of the action of INA protein and hence causes freezing point depression by adsorption inhibition[27]. At times, the presence of antifreeze proteins in food causes problems in freeze texturization[33].

With the advancements of recombinant DNA technology, the *InaA* gene from *E. herbicola* has been successfully cloned and expressed in *E. coli*[34]. Not only *InaA*, some other genes like *InaZ* of *P. syringae*, etc. associated with ice nucleation have also been cloned successfully in *E. coli*[20] and other hosts, thereby, leaving ample scope to explore their commercial value and applications in myriads of areas.

Applications of bacterial ice nucleation

Artificial snow making

Snow is a loose ice mass entrapping air inside, which is highly resistant to temperature. In general, snow is manufactured by spray freezing operation using a snowgun, which handles between 10 and 100 gallons of water per minute along with several hundred cubic feet of compressed air and the process is very energy intensive. Snow is commonly used for recreational skiing, skating and ice hockey grounds. Recently, Eastman Kodak in collaboration with Advanced Genetic Sciences, Inc. (now a part of DNA Plant Technology Corp.) has patented a product called SNOMAX which is nothing but a freeze dried, radiation sterilized powder of bacteria *P. syringae* PS 31. Each gram of freeze dried powder contains about 10^{11} ice nuclei with nucleation threshold of –5°C or warmer. The main advantages imparted by Snomax include reduction in energy consumed by snowmaking machines for each unit of snow produced and an improvement in the recreational quality of artificial snow[35]. This product has not yet been marketed in India and as it is prepared by following common fermentation technology, its cost will come around the same as already exists for freeze dried preparations of *Bacillus thuringiensis* used all over the world as a bioinsecticide. Use of this product reduces the time and temperature required, therefore, considerably improving the economics of snow making.

Freeze-texturing using INA$^+$ bacteria

INA$^+$ *E. ananas* cells can be used for freeze texturing of foods. Addition of INA$^+$ bacterial cells to isotropic aqueous dispersion of hydrogels composed of proteins and polysaccharides converts bulk of water into directional ice crystals at subzero temperature, not lower than –5°C and forms an anisotropically textured product. Slicing rightangle to the lamellae of flakes formed produces fibrous textured food. INA$^+$ bacteria inhibit supercooling of the bulk water in aqueous dispersions and hydrogels of food proteins and polysaccharides. This effect changes the ice crystallization process which improves the texture of food. This method is commonly applied to heat-sensitive foods which need texturing. INA$^+$ bacterial texturization has been successfully experimented in raw egg white, bovine blood, soybean curd, milk curd, corn starch paste, agar hydrogel, soybean protein slurries, hydrogels of glucomannan and calcium bridge glucomannan gel, etc[36]. Some of the advantages imparted by INA$^+$ bacterial texturization of food include preserving foods with high quality, saving the refrigeration cost, shortening the freezing time, improvement in quality of food especially flavour and taste, reduction in degree of super cooling, reduced initial freezing temperature, improved efficiency of production, increased freezing rate, formation of less damaging ice crystals and above all, saving of energy.

High pressure sterilized cells of INA$^+$ *X. campestris* INXC-1 have been permitted for food use in Japan. A Japanese company QPCo, Tokyo has already started commercial production of *X. campestris*. The food grade INA$^+$ bacteria must be environmentally safe, non-toxic, non-pathogenic and palatable to human beings. Generally, the use of live INA$^+$ cells in food systems is discouraged and hence, these should be killed before use by high pressure treatment or radiation[37]. But, this has a promising future as the ice nucleation gene can be cloned to edible food grade organisms like *Lactobacillus* or yeast which can be consumed directly.

Freezing foods using INA$^+$ bacteria

INA$^+$ bacteria have successfully been used for freezing of salmon fish muscle, whole fish, surmi, ice cream, agar gel, sucrose solution, fruit juices, egg white, coconut oil, sunflower oil, milk and dairy products. The application of INA$^+$ bacteria in freezing provides quick freezing, increase in nucleation temperature and energy saving. It has been reported that the INA$^+$ *P. syringae* cells can cause a whole fish to freeze consistently at –5°C within few hours, whereas up to 33% of the untreated fish do not freeze at the same temperature[38]. The total energy saving is due to the savings made during the freezing process as a result of the total freezing time being shortened and the saving made during the storage of frozen foods. For example, a frozen food plant may be able to operate at –30°C rather than at –40°C, because the use of INA$^+$ bacteria will ensure that the foods freeze at a relatively higher temperature[24]. If this plant operates at 5000 kV, the difference of electrical load between these two temperatures will be around 313 kW. Hence, the use of INA$^+$ bacteria will save a lot of energy.

Freeze drying using INA$^+$ bacteria

The cells of *E. ananas* were applied for the freeze drying of food items such as soy sauce and soybean paste, which are difficult to freeze under normal conditions. Such high salt containing food items are usually diluted for effective freezing[39]. The addition of INA$^+$ cells has made it possible to shorten the freezing times of these foods and to obtain powdered freeze-dried product with greater efficiency.

Freeze concentration using INA$^+$ cells

The cells of *E. ananas* entrapped in calcium alginate are also used for freeze concentration purpose. Generally, INA gel is placed in a desired position in liquid to be concentrated and gradually the temperature is lowered. Ice crystals formed at INA gel site along with INA gel are removed to get a concentrated product[40]. *E. ananas* IN-10 and *X. campestris* INXC-1 can be used for freeze concentration. Fresh milk, lemon juices, strawberry paste, raw egg white have been successfully concentrated using this technique[40].

BIND assay

Bacterial ice nucleation diagnostic (BIND) assay was developed by DNA Plant Technology Corporation, USA for detection of *Salmonella* in food[41]. This method uses the recognition specificity of a bacteriophage P22 and the sensitivity of the gene (*InaZ*) encoding ice nucleation protein for detection of pathogens in foods. To explain it at molecular level, *Ina* gene introduced to a bacteriophage is kept under the control of a strong promoter by recombinant DNA technology, the phage is allowed to transduce the target bacteria and the test sample is cooled to –9.5°C in presence of a freezing dye which shows fluorescent green colour at super-cooling. This indicates absence of target organisms and shows non-fluorescent orange colour at freezing, indicating the presence of target organisms, i.e. *Salmonella*. The frequency of freezing of test samples can indicate the degree of contamination. This method has been employed successfully in raw egg, milk, meat, gelatin, etc. to detect *S. typhimurium*, *S. dublin*, *S. paratyphi B*, *S. enteritidis*, *S. gallinarum* and *S. typhi*. The method is quick and shows high specificity and sensitivity and can detect even < 10 cells/ml of sample[42]. In BIND assay, non-target organisms do not interfere and it can also detect injured cells as only 100–200 INA protein molecules are required for detection. But, it has certain disadvantages, i.e. it cannot detect phage-resistant cells and also fails to detect dead cells. Moreover, as this method fully depends upon specificity of phages, it cannot be employed for detection of certain bacteria for which bacteriophage is not known.

Control of frost damage to plants

As most of the INA$^+$ bacteria are the common inhabitants of phylosphere, they cause great damage to crop plants like wheat, oat, barley, mustard, etc. by inducing ice formation on plant surfaces[43]. The economic impact of frost damage in US has been estimated to be over a billion dollars per year[44]. Several methods are now in vogue to control frost damage to plants like genetic improvement, use of protective covers, use of wind machines and heaters, application of water to plants, application of chemicals, use of antifreeze proteins, etc. of which ice-minus bacteria are gaining importance day by day.

Ice-minus bacteria are nothing but the INA$^+$ bacteria engineered genetically (*Ina* gene removed) to lose its ice-nucleating power[45,46]. Generally, ice-minus bacteria are sprayed on plant surfaces before ice-plus population develop and grow to a sufficient number to prevent the latter from colonizing. This method has given excellent results as 70–80% reduction of ice accumulation has been observed on strawberry plant[47]. But, till date Environmental Protection Agency and government have not permitted release of these organisms to nature. However, these ice-minus bacteria do not express any competitive advantage over the wild strains of ice-positive bacteria, therefore, it is difficult to control INA$^+$ population on plant surfaces in uncontrolled natural environment.

Other possible uses

Bacterial ice nucleation finds possible applications in replacement of silver iodide in artificial rain (cloud seeding and cloud precipitation); in air-conditioning for improvement of its efficiency; in refrigeration system and ice-flacking machine; processing of antifreeze protein containing foods; identification of pathogens and reduction of cold tolerance of insects in frozen grain storage[48,49]. Certain US patents concerning the use of INA protein and its related products for fluorescent immuno assay kit, treatment of hazardous waste, purification of organic chemicals have also been developed. Use of BIND assay can be extended to study the sensitivity of the bacteria to bacteriocidal agents. The initial discovery that plant surfaces shed bacterial ice nuclei in the environment was prompted by the general interest by meteorologists in ice nucleation[50]. Since then, a lot of intensive investigations have been continuing by different groups on the incidence and importance of bacterial ice nucleation in the atmosphere[51]. But till today, the meteorological significance of bacterial ice nuclei remains unproven.

Conclusion

Ice nucleating bacteria can play a significant role not only in affecting our environment but also influencing our day to day life. Although most of the possible applications involving these ice nucleation proteins are at their embryonic stages at the moment, their application and scope are bound to increase in the near future as more basic information on these wonder proteins pour in.

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Aerosol measurements at Trombay relating to the 1995 solar eclipse

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This study examines the effect of the solar eclipse on the characteristics of atmospheric environment, particularly on the aerosol concentrations at Trombay, Mumbai. Results of the measurements indicated 2–4 fold increases in the aerosol number and mass concentrations which occurred mainly in the sub-micron size range and were not noticeable above 1.0 µm. The increases occurred after a time lag of about 80 min from the beginning of the eclipse. These may be explained in terms of the combined effect of condensational growth due to increased relative humidity and a build-up of particle concentrations arising out of a suppressed level of atmospheric turbulence.

A total solar eclipse occurred on 24 October 1995. A study was undertaken to examine its effect, if any, on the characteristics of the atmospheric aerosols. The eclipse was total in Rajasthan, Uttar Pradesh and West Bengal and partial in Mumbai (72% obscurity) lasting from 0725 h (IST) to 0945 h with a peak at 0831 h. A total eclipse was last seen in India across the southern Indian peninsula on 16 February 1980. A large body of information exists on several atmospheric effects of the eclipse such as the solar radiation measurements[1–4], ozone levels[5], radon levels[6], atmospheric conductivities[7], etc. However, there exists no report of measurements on the eclipse-related behaviour of aerosols, although it is expected that cooling of the atmosphere due to reduced solar radiation intensity would result in increased aerosol levels. The need for these measurements arises from the fact that they are useful in interpreting the changes in the ground level radiation fluxes and atmospheric conductivities occurring during the eclipse. This also gives an idea of the response time of the atmospheric particles to the solar radiation changes. Besides, ambient aerosols can affect public health; from this point of view also, it is important to ascertain the change in aerosol levels, if any, during the solar eclipse.

Methodology

Several supporting measurements, viz. solar radiation variations, meteorological parameters and major pollutant levels were made, the details of which are published elsewhere[8]. However, here we emphasize the aerosol measurements as they have shown interesting changes. These were made at a height of about 10 m above the ground at the terrace of Modular Laboratory building inside Bhabha Atomic Research Centre on the day of the eclipse (24 Oct.) as well as on the preceding (23 Oct.) and succeeding days (25 Oct.) between 0700 and 1300 h. A brief description of the different monitoring instruments used and the observations made is as follows.

Mass-size distribution

The mass-size distribution measurements of the aerosols were carried out using the Quartz Crystal Microbalance Cascade Impactor (QCMCI), which is a near real-time measuring instrument operating at a flow rate of 0.24 lpm. It measures the suspended particulate matter (SPM) concentration (µg/m^3) and the mass distribution in ten size intervals with lower cut-off sizes at 0.05, 0.1, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.5 and 25 µm. Size classification is achieved by impaction on quartz crystal substrates and estimating the deposited masses on each stage by relating them to the changes in the oscillation frequencies of the crystals. The measurements were made every ten minutes.

Number-size distribution

The number size distribution measurements of the aerosols were carried out using three different instruments at every ten-minute interval. Two of them were optical particle counters (OPCs of Met-One and Malvern), while the third was the automatic condensation nucleus counter (ACNC) developed indigenously[9].

The Met-One OPC is a single particle counter based on the principle of scattering of a laser beam by particles and operating at a sampling flow rate of 2.8 lpm. It monitors the number concentration (no./cc) in six size ranges with the lower cut-off sizes at 0.1, 0.2, 0.3, 1.0, 3.0 and 5.0 µm. The size classification is achieved by relating the scattered intensity from each particle to its size. The other OPC (Malvern) is a portable instrument which monitors the number concentration in 4 size intervals with lower size cut-offs at above 0.3, 1.0, 3.0 and 5.0 µm. It essentially works on the same principle and flow rate as the Met-One OPC, but uses a laser diode as the light source.

The total particle number concentrations in the entire size range (> 0.003 μm) were monitored using the ACNC. It works on the principle of condensing water vapour on otherwise undetectably small particles and allowing them to grow in sizes at which they are detected through scattering of light.

Results

Figure 1 *a* shows the variation of the SPM concentration with time beginning at 0700 h up to 1330 h (330 min) for the three days as per the protocol. On the days preceding and succeeding the eclipse day, the variations are similar and indicate a level ranging from 10 to 80 μg/m^3. A general trend of decrease interspersed with minor peaks and troughs is seen as the day progresses. However, on the eclipse day, the behaviour is distinctly different and several phases of change are noticed. The mass levels were significantly high in the early morning (0700 h) which can be attributed to the haze that was observed that morning and then decreased rapidly to a low value at the time of the onset of the eclipse. The most noticeable aspect is the sharp increase to a value of 140 μg/m^3 beginning at 0850 h and lasting up to 1020 h. The duration of this peak (≈ 90 min) is comparable to that of the eclipse (80 min), although it manifests after a time lag of about 85 min with respect to the beginning of the eclipse. This lag is to be qualitatively expected since the aerosol behaviour is coupled to several complex atmospheric processes related to the reduction in the solar intensity and consequent cooling. After this period, the levels remained higher than those found on the other two days. A size-resolved analysis of the data (Figure 1 *b,f*) indicates that a significant part of the increases observed during the eclipse period is attributable to the particles in the 0.1–0.4 μm MMAD range. For larger sizes, this increase progressively diminishes.

Similar results are obtained for the number size distribution of aerosols. The measurements by Met-One OPC in the size group above 0.3 μm, presented in Figure

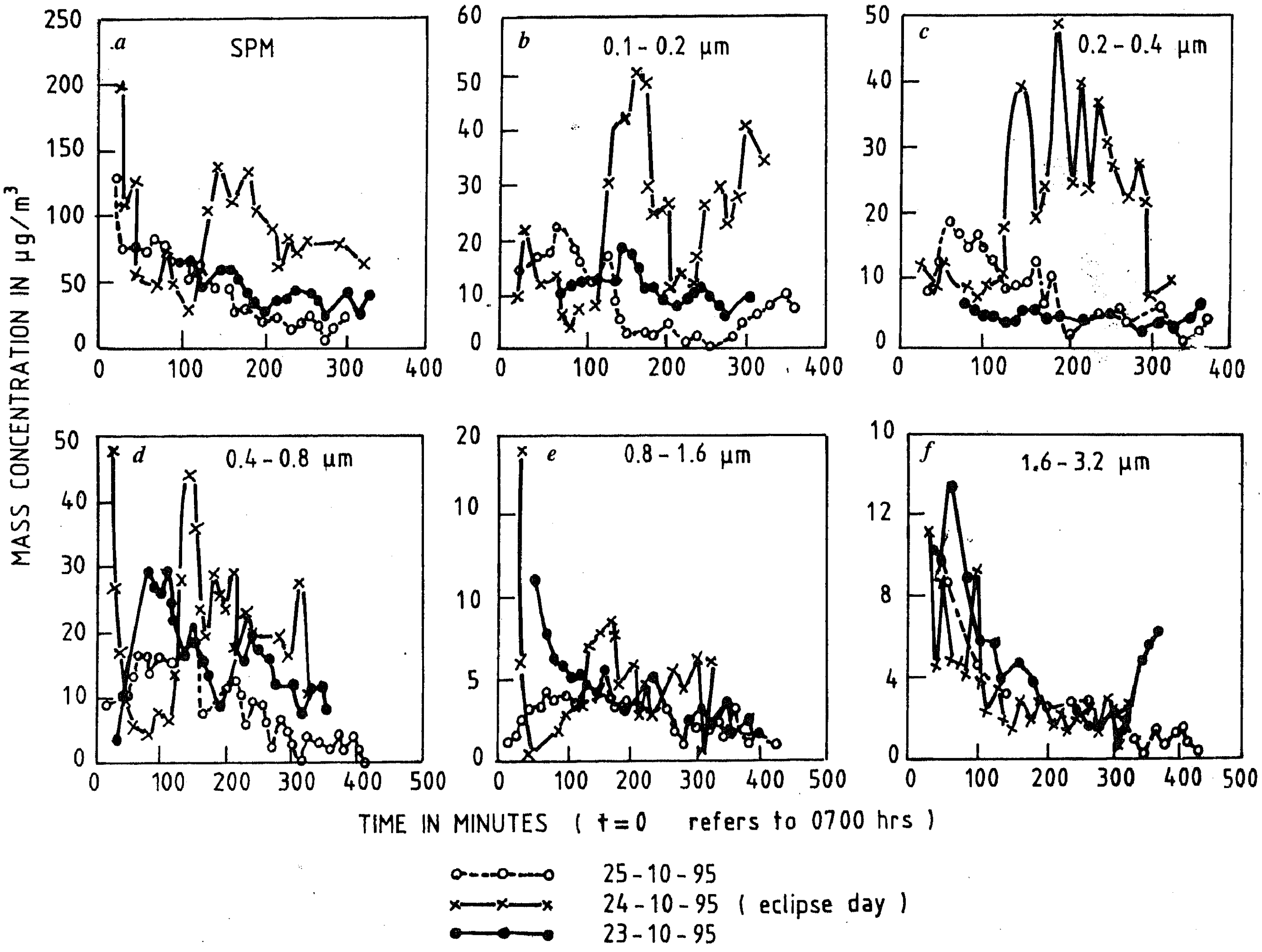

Figure 1. Temporal variation of mass concentration in different size groups as monitored by quartz crystal microbalance cascade impactor.

2 *a*, indicate that the concentration levels are low on the days preceding and succeeding the eclipse and there is a sharp peak corresponding to the eclipse period. The crest-to-trough ratio is about 3.5, similar to that observed for mass concentrations. The elevated concentrations during the haze period as well as in the post-ecliptic period are also reproduced here. Like in the case of QCM measurements, the size-resolved analysis (Figures 2 *b*, *e*) indicates a significant contribution to this increase from the size range of 0.3–0.5 μm. The results with the Malvern OPC in the size range > 0.3 μm, especially the peak number concentration observed during the eclipse period are also in conformity with those of the Met-One OPC.

The variation of the total number concentration of particles (> 0.003 μm) as measured by ACNC is shown in Figure 3. The overall trend including the concentration increase is similar to that observed with the other instruments, the main contribution in this case being from the ultrafine nuclei (< 0.1 μm) which are not recorded with the OPCs. The main difference is the appearance of a wider peak (≈ 160 min) with a greater lag time than that shown by the other instruments.

Discussion

The results indicate that an increase in the aerosol concentration (mass as well as number) did indeed occur between 0830 and 1030 h on the eclipse day which can be directly attributable to this phenomenon. The explanation, in all likelihood is contained in the time variation of the relative humidity (RH) and atmospheric turbulence profiles for that day. The temperature and humidity profiles for the eclipse day and the next day are shown in Figures 4 *a* and 4 *b* respectively. On the eclipse day, there is a definite cooling by about 2°C in the period between 0800 and 0900 h. Coincidentally, the RH was high (~90%) in the early morning hours of the eclipse day and is linked to the prevalence of the hazy conditions (which was not the case the next day). It decreased rapidly to 69% by 0800 h. Between 0800 and 0900 h, it showed an increase to a level of about 80%, consistent with the decrease in temperature. This can lead to condensation effects, especially on salt particles expected to be present in Trombay (situated on the sea coast), which manifest as increases in the number concentrations as well as in the mass concentrations. The former increase is due to the shift of very fine undetectable particles into the detection range of the OPCs and the latter includes the actual change in the mass of the particles due to water vapour condensation. It may be noted that the observed increases in concentrations (Figures 1–3) occurred after about 0840 h, but lasted much longer than the duration of humidity changes.

The observed changes will also be related to wind and turbulence conditions in the atmosphere. The wind

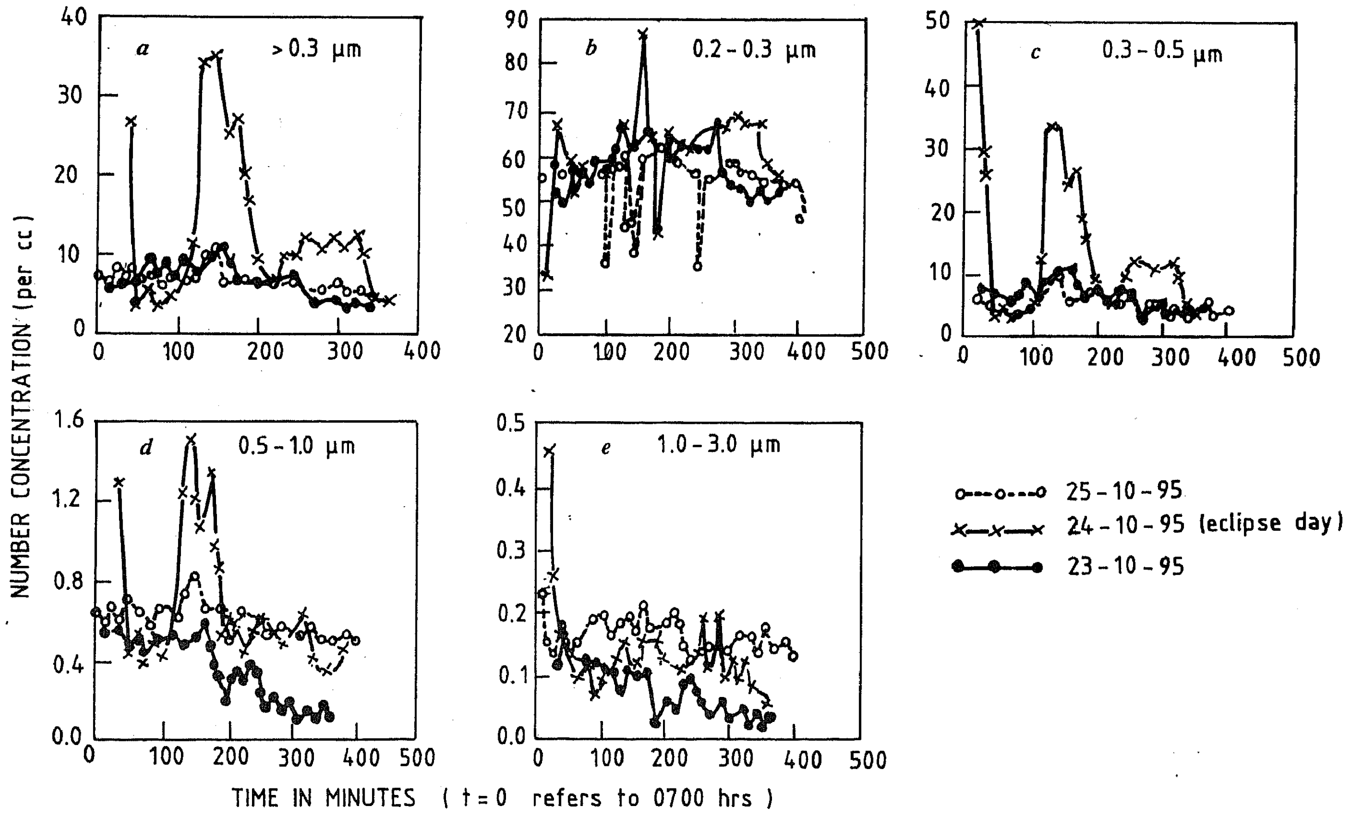

Figure 2. Temporal variation of particle number concentration in different size groups as monitored by optical particle counter.

speed, direction and standard deviation in the direction (σ_θ) are presented in Table 1 for the three days. As may be seen, both the wind speed and σ_θ were lowered during the eclipse period and calm conditions prevailed. Aerosol build-up under such conditions is a likely cause of the observed increases.

Another supportive argument for the prevalence of calm conditions and lower turbulence levels during eclipse is provided by the atmospheric back-scatter profiles up to a height of about 1000 m recorded by the SODAR (Sound Detection and Ranging) system installed at BARC. These are directly related to the thermal turbulence present in the atmosphere. The SODAR echograms for the time–height profiles showed lower back-scattered intensities (lower turbulence levels) at the ground level (below 200 m) during the eclipse period, in contrast to that on the next day during the same period. This would lead to an overall build-up of particle concentrations. The increase monitored by the ACNC (a significant part of which falls in the Aitken size range) which cannot be directly explained in terms of the humidity effects, is largely attributed to this build-up effect. A possible increase in the ground level radon concentrations as was observed during the earlier solar eclipse[6] can lead to the production of small ions. These can result in the enhancement of the concentration of radon-generated-ion-induced nuclei in the presence of water vapour that might be an additional reason for the increases observed with the ACNC.

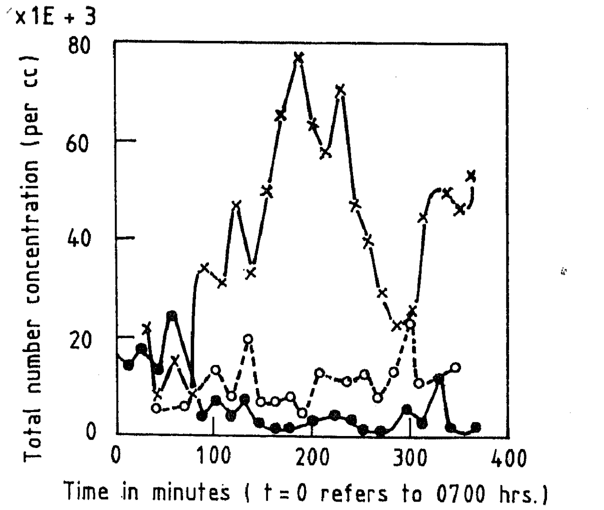

Figure 3. Temporal variation of total particle number concentration as monitored by automatic condensation nucleus counter. o– –o 25.10.95, ×—× 24.10.95 (eclipse day), •– –• 23.10.95.

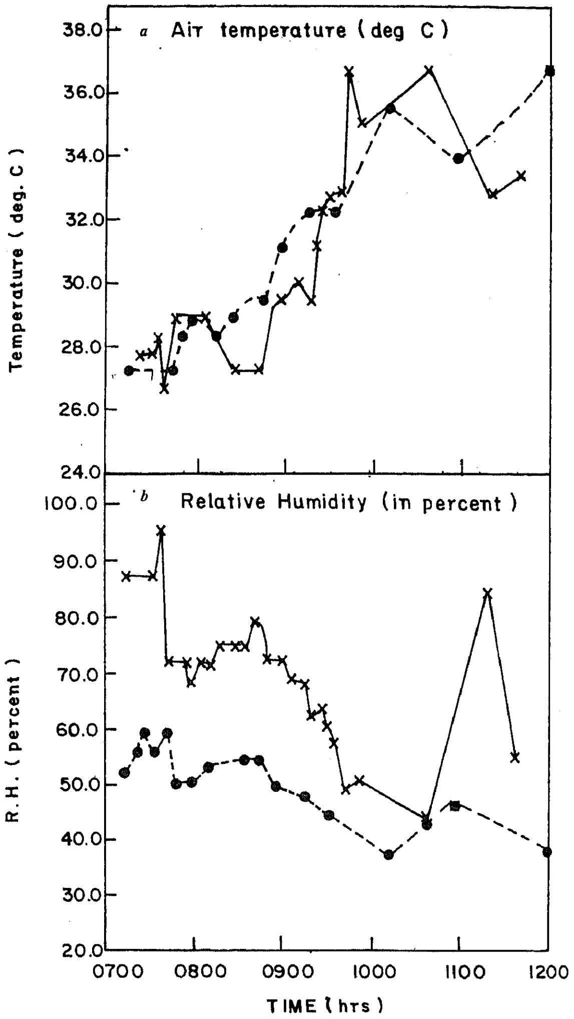

Figure 4. Temporal variation of (*a*) air temperature and (*b*) relative humidity. ×—× 24.10.95 (eclipse day), •– –• 25.10.95.

Table 1. Results of wind measurements made during this study

	23 Oct. 1995			24 Oct. 1995			25 Oct. 1995		
Time (h)	Wind speed (km/h)	Wind dir.	σ_θ	Wind speed (km/h)	Wind dir.	σ_θ	Wind speed (km/h)	Wind dir.	σ_θ
0600	3	ENE	9	5	SSE	10.5	7	NNE	9
0700	4	ENE	9	2 (C)	E	4.5	7	NE	7.5
0800	5	ENE	10.5	3 (C)	E	7	5	ENE	9
0900	7	ENE	12	3 (C)	V	V	4	V	V
1000	7	NE	–	7	ESE	10.5	5	ENE	10.5
1100	7	ENE	–	6	ESE	8.3	8	ESE	10.5
1200	8	NE	–	5	SE	9	6	ESE	13.5

V: Variable; C: Calm; σ_θ: Standard deviation in the wind direction (in degrees).

As may be seen, there was a second increase in the mass and number concentrations at about 300 min (1100 h) on the eclipse day. At the same time, we note that the temperature showed a decrease (from 36°C to about 33°C) with a corresponding rise in humidity from 50% to 80%. This suggests a possibility of repeated condensational growth due to natural changes in meteorological conditions.

An important point to be noted while comparing the size-wise results of the OPCs with those of the QCM is the differences in the interpretation of the sizes. The OPCs refer to the light-scattering diameter which for spherical particles would be the geometrical diameter. The QCM, on the other hand, groups particles according to their aerodynamic diameters, which depend on the aerosol density. As the density is not clearly known in the present case (although a value of 1.5–1.8 g/cc might be appropriate for aerosol particles), size comparisons are made only to indicate the trends. Another point is that the changes in the number concentration implies generation or build-up of particles but does not give information on their growth due to condensation. On the other hand, changes in the mass concentrations include this aspect as well.

Conclusions

There does not exist previous data on aerosol build-up from direct measurements during solar eclipse. Nevertheless, this had been earlier inferred from the lowered atmospheric conductivity measurements[7]. The present studies provide a direct confirmation of the expected increases in the aerosol concentrations due to the build-up effect. Although the changes in the temperature and relative humidity are small during the eclipse, they seem to have an important bearing on the observed increases in the aerosol concentrations. Apart from this, suppression of ground level turbulence and also possible enhanced nucleation processes induced by radon-generated ions, could be other explanations for the observed increases.

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Role of thyroidal and testicular hormones in regulation of tissue respiration in hibernating and non-hibernating species of frogs

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***In vivo* and *in vitro* effects of L-triiodothyronine (L–T_3), L-tetraiodothyronine (L–T_4), propyl thiouracil (PTU), testosterone (T) and cyproterone acetate (CA) were studied on the rate of tissue respiration in Indian streaked frog, *Rana limnocharis* (a hibernating species) and Indian skipper frog *Rana cyanophlyctis* (a non-hibernating species) maintained under natural climatic conditions during winter and summer months. The rate of tissue respiration was measured with the help of an oxygen electrode. Hormones were used in physiological doses. PTU invariably decreased respiratory rate in both the species. However, tissues of *R. cyanophlyctis* were comparatively more sensitive to thyroid hormones. Both *in vivo* and *in vitro* administration of testosterone significantly increased and *in vivo* administration of CA significantly decreased the rate of tissue respiration in both the species irrespective of seasons and temperature. These findings suggest that while the oxidative mechanisms of the two species differ in their sensitivity to thyroid hormones, endogenous thyroid hormones seem to play an important role in energy metabolism of frogs. Further, testicular hormones seem to be directly involved in the regulation of the oxidative metabolism and might be responsible for maintaining normal activity of *R. cyanophlyctis* during winter months.**

HORMONES play a major role in the regulation of the oxidative metabolism in vertebrates and ensure energy production for normal functioning of a large number of organs. Investigations over the last four decades have revealed that the energy metabolism in homeotherms is controlled primarily by thyroid hormones[1–3]. However, these hormones stimulate the metabolic rate of poikilotherms only at ecritic/high temperature (25°C or above)[4,5]. There are only a few reports regarding the role of thyroid hormones in the regulation of metabolic rate of amphibians in relation to ambient temperature[6,7]. Moreover, these studies were conducted at simulated temperatures on temperate zone amphibians maintained under laboratory conditions[8–12], and so far no attempt has been made to investigate the role of thyroid hormones in the calorigenesis of amphibians maintained under natural climatic conditions[5]. Recent studies suggest that gonadal hormones might be actively involved in the regulation of respiration in reptiles exposed to low temperatures where thyroid hormones generally do not influence the oxidative metabolism[5,13]. Notwithstanding these reports, however, there is scarcity of information on the role of gonadal hormones in the amphibian oxidative metabolism, particularly at low temperature[14,15]. Further, there are anurans which do not experience cold torpor (hibernation) even during severe cold winter months. But so far no attempt has been made to study the differences between hormonal regulation of the oxidative metabolism in hibernating and non-hibernating species of frogs with special reference to natural ambient temperature and gonadal activity[5]. Therefore, keeping in view the critical phylogenetic position of amphibians and lack of information, it was thought worthwhile to undertake a study of the role of thyroidal and gonadal hormones in the regulation of the oxidative metabolism of a hibernating and a non-hibernating species of frogs maintained under natural climatic conditions during winter and summer months.

Materials and methods

All experiments were conducted on male Indian streaked frog, *Rana limnocharis* (a hibernating species; body weight: 8–10 g; snout to vent length: 31–33 cm) and Indian skipper frog, *Rana cyanophlyctis* (a non-hibernating species; body weight: 10–12 g; snout to vent length: 35–39 mm) which are found in and around Shillong (Lat., 25°30′N; Long., 91°52′E; altitude: 1450 m ASL; minimum temperature: 0–4°C, maximum temperature: 26°C). *R. limnocharis* develops anorexia and becomes lethargic during winter months (November to January) and breeds during rainy/summer season (April to August). *R. cyanophlyctis* does not exhibit cold torpor/hibernation and remains physically and reproductively active throughout the year[16,17].

Chemicals

All the hormones and the chemicals were purchased from Sigma Chemicals, USA.

In vitro experiments

Male *R. limnocharis* and *R. cyanophlyctis* were collected from nature during December (winter, temperature: 6–14°C) and July (summer/rainy season: temperature: 14–21°C). Four frogs of each species were killed immediately after collection, and tissues (liver and skeletal muscle) were rapidly removed, rinsed in ice-cold frog Ringer's solution and stored in a freezer (– 10°C to – 15°C) for *in vitro* hormonal treatments. Storage in freezer for up to 15 days has no effect on the rate of tissue respiration. The stored tissues were used within 10 days from the date of collection for *in vitro* treatments. For treating the tissues with hormones, tissues were blotted, weighed and homogenized in ice-cold frog Ringer-phosphate buffer solution (pH 7.4) using a loose-fitting glass homogenizer. One ml of tissue homogenate (16–20 mg tissue/ml), 3.9 ml of Ringer-phosphate buffer and 0.1 ml of hormone solution (having desired concentration of hormones) were used for measuring the rate of tissue respiration. The homogenates treated with L-triiodothyronine (L–T_3) (0.6 µM), L-tetraiodothyronine (L–T_4) (0.5 µM) and testosterone (1.38 µM) were preincubated at 4°C prior to the measurement of the rate of tissue respiration using an oxygen electrode.

In vivo experiments

Male *R. limnocharis* and *R. cyanophlyctis* were collected from the field during December (temperature: 7–15°C) and July (temperature: 12–21°C) and maintained in plastic cages with mud and water. The cages were fully exposed to the natural climatic conditions. The frogs were acclimatized for 10 days. After acclimatization, the frogs of both species were separately divided into 6 groups of 4 each during winter. The first group of each species was injected only frog saline (0.1 ml) and served as control. Groups 2, 3, 4, 5 and 6 were administered L–T_4 (2 µg/frog), L–T_3 (2 µg/frog), propyl thiouracil (PTU) (1 µg/g body weight), testosterone (2 µg/frog) and cyproterone acetate (CA) (1 µg/g body weight) respectively. All the injections were given intra-muscularly at an interval of 24 h between 9 am and 10 am for four days. The frogs were provided with live earthworms as food *ad libitum*. Twentyfour hours after the last injections, frogs of each group were decapitated, tissues (liver, skeletal muscle and kidney) were rapidly removed, rinsed in ice-cold frog Ringer's solution, and stored in a freezer (– 8°C) for measuring the rate of tissue respiration with the help of an oxygen electrode. During July, after acclimatization, frogs of each species were divided into ten groups of four each. Group 1 of both the species received only saline (0.1 ml) and served as control. Groups 2, 3, 4, 5 and 6 were administered L–T_3, L–T_4, PTU, testosterone and CA respectively. The doses, mode of administration and the duration of administration of the hormones were the same as those during the winter months. Frogs of groups 7, 8, 9 and 10 were intra-muscularly injected epinephrine (2 µg/frog), norepinephrine (2 µg/frog), PTU + epinephrine (1 µg/g + 2 µg/frog) and PTU + norepinephrine (1 µg/g + 2 µg/frog) respectively at an interval of 24 h between 9 am and 10 am for four days. Twentyfour hours after the last injections, frogs were decapitated and tissues were procured, processed and stored for the measurement of respiratory rate following the procedure mentioned earlier. The stored tissues were processed within 10 days for the measurement of the rate of tissue respiration.

Measurement of the rate of tissue respiration

The rate of tissue oxygen consumption was measured with the help of an oxygen electrode (Digital Oxygen System Model 10, Rank Brothers Ltd, UK). For measuring tissue respiration, tissues were blotted, weighed and homogenized in ice-cold frog Ringer-phosphate buffer solution (pH 7.4) using a loose-fitting glass homogenizer. The polarizing voltage was kept at 0.6 V and the homogenizing medium was used as the polarizing medium. Since the preferred body temperature of most amphibians ranges between 25 and 28°C (ref. 18), temperature of the incubation chamber of the electrode was maintained at 25°C with the help of a thermostat-controlled water circulator. The homogenates were incubated in the incubation chamber of the oxygen electrode for 20 min before recording the readings. Readings for each tissue were taken for 30 min in the linear range of oxygen consumption. The rate of tissue respiration was calculated and expressed as µl O_2/mg wet tissue/hour (ref. 15). The data were tabulated and analysed using Student's *t* test[19]. A probability of less than 5% was considered to be significant.

Results

In vitro experiments

The data are presented in Table 1. *In vitro* treatments with L–T_3 and L–T_4 did not stimulate liver and muscle tissues respiration of both the species during winter and summer (Table 1). However, the respiratory rate of the tissues was significantly increased following *in vitro* treatment with testosterone during both winter and summer (Table 1).

In vivo experiments

The data are presented in Table 2. *In vivo* administration

Table 1. *In vitro* effects of thyroid hormones, propyl thiouracil and testosterone on the rate of tissue respiration of *R. limnocharis* and *R. cyanophlyctis* during winter and summer

	Rate of tissue oxygen consumption (μl oxygen/mg/h)			
	R. limnocharis		*R. cyanophlyctis*	
Treatment	Liver	Muscle	Liver	Muscle
Winter				
Control	0.88 ± 0.07*	0.61 ± 0.03	0.80 ± 0.07	0.40 ± 0.02
L–T$_3$	0.92 ± 0.09	0.71 ± 0.09	0.90 ± 0.06	0.45 ± 0.04
L–T$_4$	0.86 ± 0.02	0.60 ± 0.09	0.81 ± 0.05	0.40 ± 0.05
Testosterone	1.26 ± 0.10^{a}	0.80 ± 0.03^{b}	1.24 ± 0.08^{b}	0.81 ± 0.03^{a}
Summer				
Control	1.81 ± 0.02*	1.65 ± 0.04	1.69 ± 0.005	1.32 ± 0.04
L–T$_3$	1.98 ± 0.08	1.76 ± 0.06	1.86 ± 0.09	1.44 ± 0.07
L–T$_4$	1.82 ± 0.04	1.62 ± 0.06	1.80 ± 0.10	1.34 ± 0.04
Testosterone	1.99 ± 0.05^{b}	1.89 ± 0.06^{a}	1.86 ± 0.06^{a}	1.51 ± 0.04^{a}

*Mean ± standard error (SE); $n = 4$.
^{a,b}Differ from the respective control value: $P < 0.05$ and $P < 0.01$ respectively.
L–T$_3$ = L-triiodothyronine; L–T$_4$ = L-tetraiodothyronine.

Table 2. *In vivo* effects of hormones and inhibitors on the rate of tissue respiration in *R. limnocharis* and *R. cyanophlyctis* during winter and summer

	Rate of tissue oxygen consumption (μl oxygen/mg/h)					
	R. limnocharis			*R. cyanophlyctis*		
Treatment	Liver	Muscle	Kidney	Liver	Muscle	Kidney
Winter						
Saline	1.04 ± 0.05*	0.79 ± 0.03	0.92 ± 0.05	1.44 ± 0.05	1.12 ± 0.02	1.30 ± 0.02
L–T$_3$	1.08 ± 0.07	0.80 ± 0.01	0.98 ± 0.04	1.46 ± 0.01	1.20 ± 0.01^{a}	1.48 ± 0.04^{b}
L–T$_4$	1.05 ± 0.04	0.80 ± 0.05	0.96 ± 0.03	1.44 ± 0.005	1.19 ± 0.03	1.45 ± 0.08
PTU	0.87 ± 0.03^{a}	0.65 ± 0.04^{a}	0.69 ± 0.05^{a}	1.20 ± 0.06^{a}	0.76 ± 0.12^{a}	0.96 ± 0.09^{a}
T	1.31 ± 0.07^{a}	0.97 ± 0.06^{a}	1.15 ± 0.04^{a}	1.71 ± 0.05^{b}	1.22 ± 0.03^{a}	1.61 ± 0.09^{a}
CA	0.74 ± 0.07^{a}	0.64 ± 0.05^{a}	0.76 ± 0.03^{a}	1.18 ± 0.07^{a}	0.96 ± 0.03^{b}	0.96 ± 0.04^{c}
Summer						
Saline	2.05 ± 0.07	1.44 ± 0.09	1.82 ± 0.03	2.01 ± 0.08	1.36 ± 0.06	1.72 ± 0.02
L–T$_3$	2.10 ± 0.07	1.52 ± 0.03	1.93 ± 0.05	2.17 ± 0.03	1.44 ± 0.06	2.02 ± 0.02^{b}
L–T$_4$	2.04 ± 0.02	1.45 ± 0.05	1.84 ± 0.06	2.12 ± 0.04	1.42 ± 0.08	1.96 ± 0.05^{a}
PTU	1.67 ± 0.03^{c}	1.20 ± 0.01^{a}	1.70 ± 0.03^{a}	1.75 ± 0.03^{a}	1.16 ± 0.04^{a}	1.51 ± 0.05^{b}
T	2.24 ± 0.03^{a}	1.80 ± 0.02^{b}	1.99 ± 0.05^{b}	2.39 ± 0.11^{a}	1.60 ± 0.07^{a}	2.04 ± 0.07^{b}
CA	1.70 ± 0.05^{b}	1.29 ± 0.07	1.71 ± 0.03^{a}	1.72 ± 0.05^{a}	1.06 ± 0.09^{a}	1.52 ± 0.04^{b}
EP	2.40 ± 0.10^{a}	1.88 ± 0.07^{b}	2.20 ± 0.07^{b}	2.50 ± 0.14^{a}	1.81 ± 0.03^{c}	2.14 ± 0.09^{b}
NE	2.60 ± 0.04^{c}	1.94 ± 0.06^{b}	2.27 ± 0.03^{c}	2.54 ± 0.12^{a}	1.90 ± 0.07^{c}	2.30 ± 0.10^{c}
PTU + EP	2.09 ± 0.04^{f}	1.58 ± 0.10^{d}	1.92 ± 0.09^{d}	2.10 ± 0.05^{d}	1.42 ± 0.07^{e}	1.85 ± 0.06^{d}
PTU + NE	2.15 ± 0.07^{e}	1.50 ± 0.01^{f}	1.99 ± 0.05^{e}	2.29 ± 0.09	1.49 ± 0.11^{d}	1.93 ± 0.08^{d}

*Mean ± standard error (SE), $n = 4$.
^{a,b,c}Differ from saline treated control group: $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
^{d,e,f}Differ from respective EP or NE treated group: $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively.
CA = cyproterone acetate; EP = epinephrine; NE = norepinephrine; PTU = propyl thiouracil; L–T$_3$ = L-triiodothyronine; L–T$_4$ = L-tetraiodothyronine, T = testosterone.

of L–T_3 and L–T_4 had no significant effect on the respiratory rate of tissues in *R. limnocharis* during winter and summer (Table 2). However, L–T_3 administration in *R. cyanophlyctis* significantly stimulated the respiratory rate of muscle and kidney during winter and only of kidney during summer. Unlike L–T_3, *in vivo* treatment with L–T_4 had no effect on the respiratory rate of tissues of *R. cyanophlyctis* during winter and summer except kidney respiration which was stimulated during the summer (Table 2). Administration of PTU invariably and significantly decreased the respiratory rate of all tissues in both the species during summer as well as during winter seasons (Table 2).

In vivo administration of testosterone significantly stimulated and CA significantly decreased the respiratory rate of all the tissues (except of muscle in *R. limnocharis* during summer) in both the species during both summer and winter seasons. CA-induced decrease in the respiratory rate of muscle in *R. limnocharis* during summer was found to be marginally insignificant (Table 2).

In vivo administration of epinephrine (EP) and norepinephrine (NE) significantly stimulated the respiratory rate of tissues in both the species during summer (Table 2). However, when EP and NE were administered in combination with PTU during summer, the stimulatory effect of the catecholamines on tissue respiration was significantly reduced compared to the respective groups which received only catecholamines (Table 2).

Discussion

The thyroid hormones did not stimulate the respiratory rate of tissues of *R. limnocharis* which undergoes hibernation/cold torpor during winter months irrespective of seasons, temperature and mode of treatment. However, *in vivo* administrations of L–T_3 and L–T_4 were found to stimulate the respiratory rate of muscle and kidney of *R. cyanophlyctis* (which remains active throughout the year) during winter and summer respectively (Table 2). It seems that tissues of the two species differ in their response to calorigenic action of thyroid hormones. Further, *in vivo* administration of PTU (a goiterogen which blocks synthesis of thyroid hormones) significantly decreased tissue respiration in both the species during both winter and summer months (Table 2). Thus, there is a possibility that the endogenous levels of T_3 and T_4 are actively involved in the regulation of the oxidative metabolism in both the amphibian species during both summer and winter months. There are reports that exogenous thyroid hormones significantly influence intermediary metabolism of poikilotherms exposed to low temperature[20,21]. In mammals exposed to low temperature, catecholamines regulate energy metabolism, and thyroid hormones reportedly support the non-shivering thermogenesis by potentiating the calorigenic action of catecholamine hormones[22–24]. Therefore, there is a possibility that endogenous thyroid hormones might be involved in maintaining the metabolic rate indirectly by potentiating the calorigenic action of catecholamines. This assumption is strongly supported by significant reduction in the stimulatory effect of EP and NE on tissue respiration when the catecholamines are administered with PTU compared to effects of catecholamines alone during summer (Table 2). The observed significant reduction in the calorigenic action of catecholamines in the presence of PTU might be due to decreased production of thyroid hormones and/or due to inhibition of deiodination of T_4 to T_3 (ref. 3). Potentiation of the calorigenic action of catecholamines in frogs by thyroid hormones seems to be of evolutionary significance. In reptiles also, thyroid hormones reportedly potentiate the metabolic effects of catecholamines at low temperature[25,26]. On the basis of these findings it can be suggested that for evaluating the calorigenic role of thyroid hormones in any amphibians/poikilotherms, use of goiterogens should be preferred over the use of exogenous thyroid hormones. In *R. cyanophlyctis*, comparatively higher tissue sensitivity to thyroid hormones might be associated with higher levels of physical and/or physiological/metabolic activity[5]. Thyroid hormones are reported to stimulate activities of several oxidative enzymes in fish and reptiles exposed to preferred body temperature[27–30].

Gonadal hormones have been reported to be directly involved in the regulation of the oxidative metabolism in several species of reptiles[2,5,13,31,32] and birds[33–35]. In the present study the rate of tissue respiration was invariably and significantly stimulated by testosterone in both the species in all the experiments. Further, in both the species *in vivo* administration of CA (a blocker of androgen receptors) significantly decreased the respiratory rate of tissues irrespective of seasons and ambient temperature. It, thus, seems that the observed decrease in tissue respiration following CA administration might be due to inability of endogenous androgens to bind to their specific receptors already occupied by CA. *In vitro* calorigenic action of testosterone strongly suggests that, as in reptiles[5,13], androgens are also directly involved in the regulation of the oxidative metabolism of amphibians. It is important to mention that during winter while spermatogenesis is arrested in *R. limnocharis*, in *R. cyanophlyctis* spermatogenesis has been reported to occur throughout the year with slight depression during winter months[16,17]. Thus, there is a probability that a comparatively higher level of androgens (as indicated by spermatogenesis) might be responsible for maintaining higher levels of metabolic rate of tissues and physiological activity in *R. cyanophlyctis* as compared to *R. limnocharis*. Direct involvement of testicular hormones in the oxidative metabolism of amphibians (present study), reptiles[5] and birds[1,33-35] suggests that androgens might be playing a dominant role in regulation of the metabolic rate in sub-mammalian vertebrates.

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Biological control of sheath blight of rice: Induction of systemic resistance in rice by plant-associated *Pseudomonas* spp.

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In previous studies on the biological suppression of fungal diseases of rice with plant-associated rhizosphere bacteria we had reported that production of antifungal antibiotic (afa) by the bacterium is the major mechanism involved. In this study we report that the induction of systemic resistance in rice by strains of pseudomonads leads to suppression of sheath blight. A molecular tracking system (*lacZY* gene) was used to engineer strains of *Pseudomonas fluorescens* 7-14 and *P. putida* V14i. We used the *lacZ* expression as tracking system to monitor the survival and migration of the biocontrol agents. In spite of the absence of bacteria on plant surfaces, the endophytic bacteria present in the stem led to suppression of rice sheath blight caused by *Rhizoctonia solani* up to 18%.

THE application of beneficial pseudomonads as agents for biological control of plant diseases in agriculture is emerging as a potential alternative to the use of chemical fungicides. Evidence for mechanisms of biological control of pathogens cannot be exemplified by a single factor. The known mechanisms include, competition, production of antibiotics, siderophores, hydrogen cyanide, phytohormones, enhancement of nutrient uptake by the plant, extracellular enzymes, nitrogen fixation, enzyme synthesis to regulate the level of plant ethylene, and other substances[1–8].

Recent investigations on mechanisms of biological control by plant growth-promoting rhizobacteria (PGPR) suggest that these strains protected plants from various pathogens by inducing a systemic resistance (ISR) in plants[9–14]. The evidence for involvement of pseudomonads in ISR was observed when lipopolysaccharides extracted from the outer membrane of *P. fluorescens* caused more accumulation of phytoalexins, increased amount of salicylic acid, phenolics and pathogenesis-related (PR) proteins[9,11,15–17]. Fluorescent pseudomonads have the capacity to exist inside plants endophytically in different host plants[12,18–20]. Previous research carried out also shows that pseudomonads have the potential for the suppression of rice diseases[5,21–24]. The objective of this study was to assess whether the rhizosphere pseudomonads could cause ISR to a fungal disease when applied as infiltration, and to monitor the migration and survivability of genetically engineered strains in the host plant.

Wild type strains of *Pseudomonas putida* V14i and *P. fluorescens* 7–14 used in this study were previously isolated from the rice rhizosphere[21,24]. *Escherichia coli* CE1 was obtained as a gift from R. Jefferson, CAMBIA, Australia. The sheath blight pathogen *Rhizoctonia solani* was isolated from infected rice tissues. LacZY gene cassette was inserted into the chromosome of recipients (7–14, V14i) by a disarmed Tn-7 lacZY transposon through triparental mating[25]. The genetically engineered strains of 7-14gal and V14igal and their constitutive expression of lacZY was stable over 50 generations and were recovered in M9 agar which contains 1% lactose, X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) and antibiotics (100µg/ml of rifampicin [rif] and ampicillin [amp] for 7-14gal strain and 100 µg/ml of streptomycin [stp] and amp for V14igal strain).

Bacterial strains of 7-14gal and V14igal were grown in M9 at 27°C for 24 h. *E. coli* CE1 strain (negative control) was grown in Lauria Bertani (LB) agar plates at 37°C. These were harvested separately, washed twice with sterile water by centrifugation at 5000 *g* for 10 min and the cell suspension was adjusted to 0.1 OD at 600 nm to obtain 10^9 cfu/ml. Twenty-day-old rice seedlings (cv. IR50) were bacterized by infiltration with 1,2 and 5 µl of bacterial cells with a microsyringe at approximately 2 cm above the soil surface. For *E. coli* CE1, only 2 µl was infiltrated about 2 mm into the stem. In another set of experiment, plants were inoculated by beading needles that were dipped in the bacterial suspension and were slightly brushed into the stem following the method reported earlier by Chen *et al.*[18]. Plants that received 2 µl of sterile water and also uninoculated plants served as controls. The experiments were conducted twice with three replications under greenhouse conditions.

The bacteria were also passively introduced by seed treatment, root-dip and foliar spray onto rice foliage with a bacterial suspension (10^9 cfu/ml) in 1% carboxymethylcellulose (cmc). For seed treatment, bacteria were applied to surface sterilized IR50 rice seeds and were incubated overnight. Excess amount of fluid was drained and it was observed that seeds had 10^9 cfu/g. The treated seeds were sown in pots containing sterile soil. Root dipping was carried out on 21-day-old rice seedlings that were uprooted, rinsed gently in sterile water, and roots were dipped for 30 min in the respective bacterial suspensions before transplanting into sterile soil. When the bacterial suspension was applied also as foliar spray on 15-day-old rice seedlings (IR50), the treatment was made until minute droplets appeared on both surfaces of the entire leaf. Rice seedlings that received plain cmc solution as spray served as control.

The survival of bacteria was monitored by removing 2 cm sections of the rice stem (1 cm above and 1 cm below the point of infiltration). To assess the movement of bacteria from the point of infiltration, 2 cm long

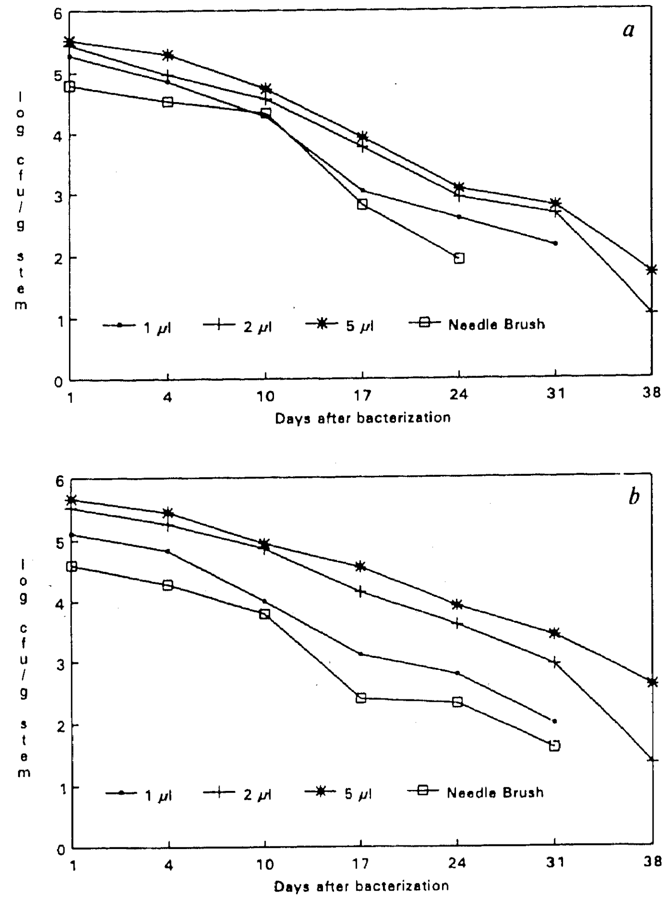
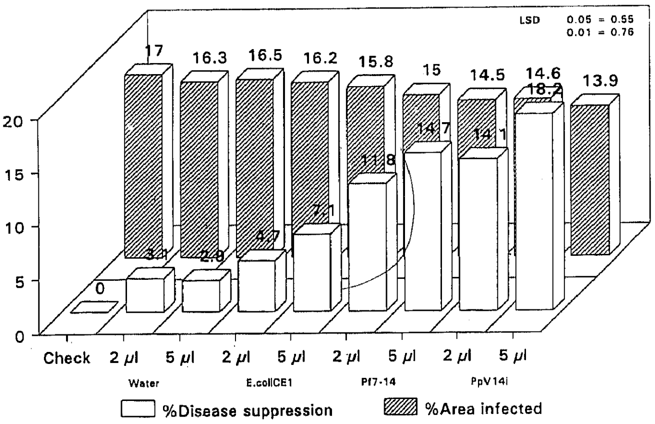


Figure 1. Survival of lacZY-marked bacterial strains after infiltration and needle brush application into the rice (cv. IR50) stem. (*a*), *P. putida* V14igal; (*b*), *P. fluorescens* 7-14gal.



Each value is mean of three replications

Percent area infected = Lesion length/plant height x 100

Percent disease suppression = 100 - treatment/control x 100

Figure 2. Suppression of rice sheath blight caused by *Rhizoctonia solani* in plants by plant associated *Pseudomonas* strains of bacteria by the induction of systemic resistance. Disease incidence in *R. solani* infected controls is assigned a value of 100%.

section was also sampled (1.5–3.5 cm [I stage] and 4–6 cm [II stage]) above the point of infiltration. The samples were weighed, rinsed for 5 min in running tap water and then were soaked for 2 min in 2% sodium hypochlorite solution. Subsequently, they were washed thrice in sterile water and were triturated in 1 ml of 0.01 M phosphate buffer (pH 7.1). Plants were sampled at 1, 4, 10, 17, 24, 31 and 38 days after the bacterial infiltration. Endophytic presence of bacteria was assessed in 1 g of rice roots removed from 10 and 15-day-old seedlings that received seed treatment or root-dip treatment or from 1 g of leaf sample in the case of foliar spray (5 and 10 days after bacterization) treatment. Appropriate serial dilutions of the triturated samples were plated on to triplicate agar plates of KB (King's B) with X-Gal with or without cycloheximide (40 µg/ml) for 7-14gal and V14igal strains. Samples that received *E. coli* CE1 strain were plated on LB agar with X-Gal and samples of untreated checks were plated on KB/LB agar plates. After 48 h of incubation, the blue coloured bacterial colonies were counted (7-14gal and V14igal were also observed under UV lamp at 365 nm for their fluorescent character) and their cfu/g of tissue was determined.

A field experiment for the systemic suppression of rice sheath blight (ShB) was conducted by planting surface sterilized rice (IR50) seeds in a randomized complete block design. Rice seedlings of 20–22-day-old were infiltrated with 2 µl and 5 µl of 10^9 cfu/ml of 24 h grown wild type 7-14, V14i and CE1 strains. Control plants received plain sterile water or did not receive any treatment (untreated checks). One day after the bacterial treatment, the plants were inoculated with *R. solani*. Inoculum of *R. solani* has been described previously[23] and was applied at the rate of half a teaspoon per hill close to the sheath portion of the rice plants. ShB severity rating was recorded on the 10th day after *R. solani* inoculation from randomly selected 10 rice plants per plot (0.5 × 0.5 m)/treatment. From these assessments, per cent disease incidence and per cent disease control were determined by statistical analysis.

The data on the survival of the two marked strains of *P. putida* V14igal and *P. fluorescens* 7-14gal are presented in Figure 1 *a*, *b*. These data show that bacteria survived up to day 38 when 2 µl or 5 µl aliquots were infiltrated. Whereas bacteria applied in a needle brush did not survive beyond 24 days (V14igal) or 31 days (7-14gal) after the treatment. The *E. coli* CE1 was detected up to 10 days after infiltration or needle brush treatment. In water treatment or nontreated control tissues, blue-coloured colonies did not develop.

Upward migration of the marked strains of V14igal and 7-14gal occurred up to 6 cm (stage II) away from the point of infiltration in the rice sheath or stem (Table 1). Beyond this point the stem and the leaves did not

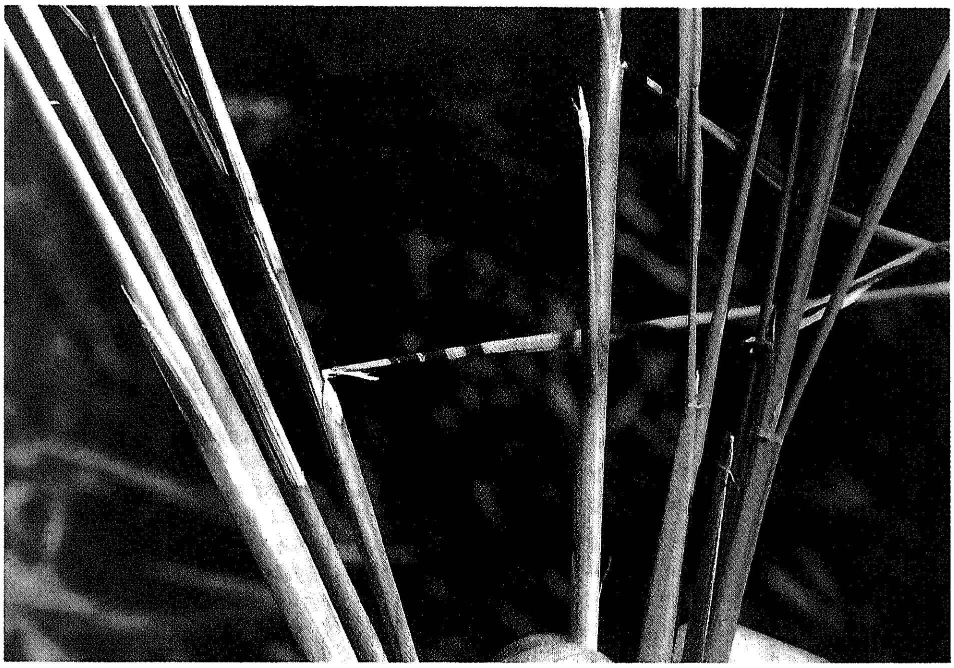


Figure 3. Reduction of sheath blight in IR50 rice plants due to the induction of systemic resistance by *Pseudomonas putida* strain. *Right*: Plants infiltrated with 5 µl of PpV14i strain; *Left*: Nontreated control plants infected with *R. solani*.

Table 1. Migration of *Pseudomonas putida* V14igal, *P. fluorescens* 7-14gal and *Escherichia coli* CE1 in the rice stem

Bacterial strain	Aliquot of inoculation used (µl)	Sampling stage	Mean log cfu/g of stem tissue recovered after (days)				
			1	4	10	17	24
V14igal	1	I	4.90[a]	3.89	3.11	ND[b]	ND
	1	II	4.71	3.67	2.38	ND	ND
	2	I	4.95	4.08	3.45	1.94	ND
	2	II	4.79	3.69	2.57	ND	ND
	5	I	4.98	4.58	4.08	1.36	ND
	5	II	4.86	3.99	3.11	1.04	ND
	NB[c]	I	3.94	3.23	1.68	ND	ND
	NB	II	2.94	1.72	ND	ND	ND
7-14gal	1	I	4.88	3.73	2.91	ND	ND
	1	II	4.71	3.51	1.95	ND	ND
	2	I	4.96	3.74	3	1.43	ND
	2	II	4.78	3.59	2.46	ND	ND
	5	I	5.08	4.58	3.43	2.92	2.54
	5	II	4.86	3.99	2.18	ND	ND
	NB	I	3.96	3.32	ND	ND	ND
	NB	II	3.04	1.85	ND	ND	ND
CE1	2	I	3.96	3.15	1.66	ND	ND
	2	II	2.99	1.11	ND	ND	ND
	NB	I	1.83	ND	ND	ND	ND
	NB	II	ND	ND	ND	ND	ND

[a] Each figure is a mean of 3 replications.
[b] Not detected.
[c] Needle brush application.

have the cells of the marked strains. However, below the point of infiltration these bacteria were detected at 57 cfu/g tissue (V14igal) and 39 cfu/g tissue (7-14gal) from day 10 after infiltration.

From the results there is no indication for the endophytic presence of the marked strains of bacteria in roots of seedlings that were raised from bacteria-treated seeds. There was also no endophytic presence of bacteria in leaf sample of rice seedlings that received foliar sprays of V14igal and 7-14gal. Bacteria applied as a root-dip were detected at 23 cfu/g root in V14igal treatment.

Figure 2 shows that *Pseudomonas* strains (V14i and 7-14) present in the rice stem reduced the sheath blight incidence by 18 and 15% in comparison with infected control, which is taken as 100% infection. Yet these bacteria were not present on the surface of the sheath or pseudostem. In spite of the absence of bacteria on surface of the rice plant there is evidence for pathogen suppression by bacteria (Figure 3). This is most likely due to the induced systemic resistance triggered by the endophytic bacteria introduced into the rice stem. The results indicate that there is a minimal reduction of ShB even with *E. coli* CE1 or by water infiltration. This might be due to the response of plants to lipopolysaccharides or other defence-inducing signal factors. This is the first report on the induction of induced systemic resistance (ISR) in rice by the rhizosphere-bacteria. Our future studies will be directed to analysing the nature of the defence substances (phytoalexins, salicylic acid or other PR proteins) involved in the induced systemic resistance in rice.

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Litomosoides carinii and *Acanthocheilonema viteae* in rodents: The influence of nonspecific immunosuppression on establishment of filarial infection

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Prolonged incubation period and poor establishment rate of adult filarial parasites in experimental host have jeopardized much of laboratory investigational activities. Study was therefore planned with the objectives of shortening the prolonged incubation period of filarial infection and enhancing the establishment rate of macrofilariids with high peripheral microfilaraemia in host. It was observed that cortisone treatment in rodents at 10 mg/kg, subcutaneously from day –7 to day + 7 in two courses at week's interval, not only reduced the prepatent period of both *Litomosoides carinii* and *Acanthocheilonema vitae* infections by 10 days but also increased the adult worm recovery to a significant extent. Microfilarial level remained higher in cortisone-treated animals throughout the observation period. It was also interesting to note that length of adult male and female filariids (*A. viteae*) recovered from treated animals was significantly more in comparison to controls. The carbohydrate metabolism of recovered parasites from cortisone-treated hosts remained unaltered and thereby, remained suitable for various experimental purposes.

THE influence of immunosuppression of host on establishment of parasite infection is well documented[1–5]. Treatment with immunosuppressant leads to increased growth rate and size of parasites by counteracting the host's natural resistance towards parasite establishment mechanism. The present study was therefore designed with the objectives of lowering the prolonged prepatent period and increasing the intensity of microfilaraemia through higher establishment rate of adult filariids by suppressing the host's immune system with cortisone, an immunosuppressant[6–10]. The study was also extended to assess the metabolic status of recovered adult filariids from cortisone-treated animals to ascertain their physiologic status.

Two rodent filarial infections, *Litomosoides carinii* in cotton rat (*Sigmodon hispidus*)[11,12] and *Acanthocheilonema viteae* in *Mastomys coucha*[13,14] were used in the study.

Hydrocortisone (Sigma) was used initially at different dose levels (5, 10 and 20 mg/kg, s.c.) and courses (5 or 10 consecutive days or in two courses with a week's interval) in cotton rat and mastomys to determine the optimum dose level to bring about desired immunosuppressive effect as revealed by performing macrophage migration test[15,16]. The dose of 10 mg/kg × 5 days followed by another course preceded by a gap of one week was found optimum. Microfilaraemia was monitored till 75 of infective exposure of all treated and control animals by examining 5 cmm of tail blood. Animals were sacrificed on day 75 and worm burden was ascertained according to the method of Chatterjee *et al.*[12]. Length of recovered male and female parasites was also measured. The contents of uteri of female parasites were examined for any abnormality in developmental stages and microfilariae. The glucose metabolism[17–19] of parasites recovered from cortisone-treated hosts was measured by assessing *in vitro* uptake and lactic acid production capabilities.

In cortisone-treated animals, microfilariae of *L. carinii* and *A. viteae* appeared initially in peripheral blood of some animals on day 45 of L_3 exposure, i.e. 10 days earlier than control animals, as monitored by blood examination for microfilaria at 5 days interval since day 30 of L_3 exposure. The per cent positivity for microfilariae on day 45 was 97.5% in case of *A. viteae* and 66.0% in case of *L. carinii*-infected animals. On day 50, all the cortisone-treated groups of animals were microfilariae-positive. The intensity of microfilaraemia in treated animals was higher ($P < 0.001$) throughout the observation period (Figures 1 and 2). Per cent recovery of adult *A. viteae* was also higher (70%) in comparison to controls (32%) (Figure 2). In case of *L. carinii* infection, the number of adult parasites recovered from treated hosts was more (28 ± 3.5) in comparison to control animals (18 ± 3.0) (Figure 1). All female parasites (both *A. viteae* and *L. carinii*) contained normal and active developmental stages in the uteri. It was

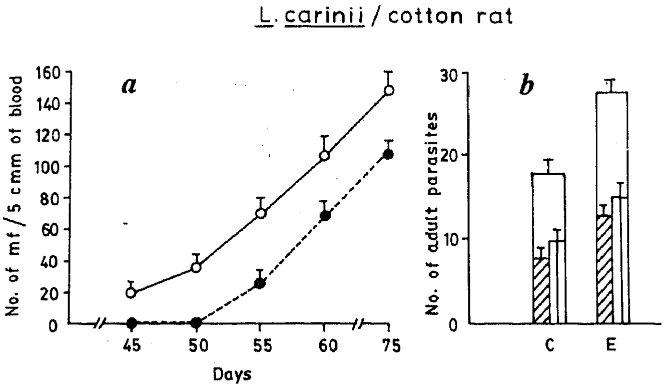

Figure 1. Microfilaraemia (*a*) and adult worm recovery (*b*) from cotton rats infected with *L. carinii*. o– – –o Cortisone treated; •– – –• Untreated control. ▨Male, ▥ Female, □Total. E, Experimental; C, Control.

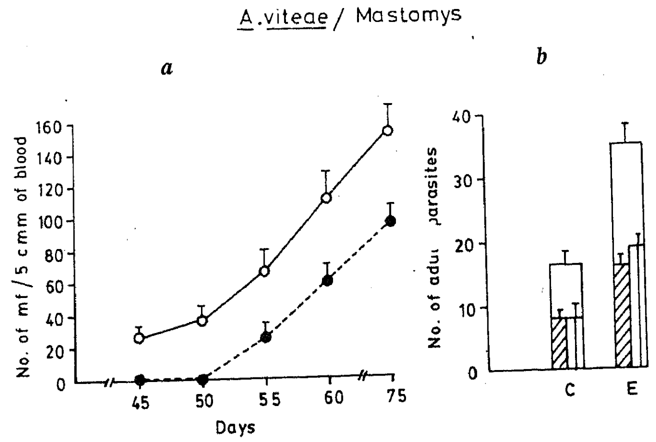

Figure 2. Microfilaraemia (*a*) and adult worm recovery (*b*) from Mastomys with *A. viteae* infection. ○---○ Cortisone treated; ●---● Untreated control. ▨ Male, ▥ Female, ☐ Total. E, Experimental; C, Control.

interesting to observe that adult male and female *A. viteae* recovered from cortisone-treated animals were longer in length than their counterparts of control animals. Lengths of female and male parasites were 8.3 ± 3.0 and 3.4 ± 0.24 cm respectively whereas worms from untreated animals were respectively 5.4 ± 3.0 and 2.7 ± 0.22 cm. No such difference in length of *L. carinii* adults could be detected when recovered from cortisone-treated animals.

The glucose uptake of adult *L. carinii* and *A. viteae* from control animals was respectively 0.64 ± 0.06 and 0.49 ± 0.10 μmole/mg worm which was well comparable to worms obtained from their respective cortisone-treated hosts. Similarly there was no difference in the level of lactate production in cortisone-exposed parasites of both the species.

Our present study with experimental filarial infections using cortisone in rodent hosts indicates that nonspecific immunosuppression can promote significantly the per cent establishment rate of adult filariids, increase in size of worms and even causes reduction in the prolonged prepatency period. Hydrocortisone is well known to interfere with a variety of intra- and extra-cellular activities of mononuclear phagocytes, diminish monocyte chemotaxis and inhibit migration of leucocytes[20]. The decisive role of T-cell activity in establishment of helminth infections has been demonstrated earlier using athymic mice[21,22] or by transfer of immunity by T-lymphocytes[23,24] or hydrocortisone, a well-known T-cell suppressant[25]. Nevertheless, timing for application of immunosuppressor is important in case of filarial infection in which various developing forms take different time periods for maturity depending upon the species of parasite as well as host and the total incubation period may vary from 2 to 8 months. Keeping these in view, the course of treatment of cortisone is broadly divided into two time periods coinciding with the stages of larval forms, i.e. from L_3 to preadult stages so that all the developing forms may take the advantage of immunosuppressive state of the host. Thus, based on larval development cycle of *L. carinii* and *A. viteae*[26], two courses of cortisone, each of five days duration were administered to produce sustained immunosuppressive effect during the course of establishment period of parasites.

Both the species of parasites showed significantly higher per cent establishment rate in their respective hosts and in one of the species (*A. viteae*), the average length of females ($P < 0.001$) and males ($P < 0.01$) was also significantly higher than their counterparts from untreated control mastomys. Moss[2] has demonstrated that *Hymenolepis microstoma* in mice treated with cortisone grows larger than in control mice, and suggested that the increased growth of worms may be due to the combined effects of immunosuppression and growth promoter, which may be a hormonal growth-stimulating factor.

It is interesting to mention that whereas nonspecific immunosuppression of host helped in better establishment rate of adult parasites, immunostimulation on the contrary reverses the situation[6,7]. Thus, immunomodulation whether suppressive or stimulatory has in general a significant role to play in establishment of filarial infection in host.

The two other parameters as observed to be altered in both *L. carinii*/cotton rat and *A. viteae*/Mastomys systems following cortisone treatment are lowering of prepatency period of 10 days and increased intensity of microfilaraemia. Lowering of prepatency period appears to be due to increased growth rate of larvae in immunosuppressed host whereas higher intensity of microfilaraemia in cortisone-treated animals happens to be due to increased worm population.

Glycolysis is the major energy-generating pathway and both *L. carinii* and *A. viteae* recovered from cortisone-treated hosts, rapidly absorbed glucose from the medium and lactate was synthesized as the major end-product. Estimation of glucose uptake and lactate production of filariids showed parasites to be metabolically normal.

In conclusion, it may be stated that nonspecific immunosuppression of experimental host leads to lowering of prepatency period, higher intensity of microfilaraemia and increased population of physiologically normal filarial parasites. The data shown in this study would be of great help in laboratory maintenance of filarial infection and for meeting the growing demand of filaria parasite material for various experimental purposes.

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Block rotation in a part of eastern Dharwar craton of South India – A suggestion from aeromagnetic data interpretation

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Deformation in the brittle part of the earth's crust often gives rise to faults. It is believed that these faults tend to form conjugate fracture systems, and the rigid crustal blocks bounded by these fractures rotate about horizontal axes in the event of dip slip and about a vertical axis in the strike slip environment. Recent studies in the granite–greenstone terrain of a part of eastern Dharwar craton in South India (3.4–2.5 Ga) have suggested a tectonic environment in which the cross cutting NW, NE and ENE trending faults systems divided the crust into a mosaic of rigid crustal blocks which might have been rotated. Analysis of the aeromagnetic data of a part of this area suggests that (i) the NW–SE trending shears have strike-slip component, (ii) the rigid crustal blocks have rotated through an angle of 8.5° clockwise and (iii) the depth of the decoupling zone beneath the strike-slip faults is estimated to be 3.0 km, i.e. it is in the upper crustal level.

UNDER a variety of tectonic conditions the brittle part of the earth's crust is often prone to deformation. This deformation generally gives rise to faults. Simultaneous translation and rotation of fault-bounded crustal fragments, which are governed by kinematic constraints, are widely reported in varied geological and tectonic settings[1–4]. Fault-bounded crustal blocks rotate about vertical axes, where a fault slip has a strike parallel component[1,5,6], and on horizontal axes where dip slip occurs. Faults also rotate under continued deformation. These block rotations may occur (i) in discrete shear zones, (ii) zones of distributed shear probably caused by oblique subduction, (iii) during accretion, (iv) due to oroclinal bending, and (v) in overthrust faulting[2]. Such rotation may take place in different scales, starting from an outcrop to regional scale[5,7,8]. Recent studies on the granite–greenstone terrain of a part of Eastern Dharwar craton of south India, based on Landsat, aerial photos, age relationships of the structures such as faults, lineaments, dykes, etc. and on field evidences, suggested that tectonically this area is a composite mosaic of crustal blocks of various dimensions. These have rotated about vertical axes in geological time in response to various tectonic processes[9]. Several workers have reported palaeomagnetic, seismic and structural evidences in support of block rotation[2,4,10–17].

The angles through which the blocks can rotate are, in some cases 15–20°, and in some other cases the horizontal rotations are reported to be 70° and even more between the two parallel faults in strike slip environment.

Aeromagnetic data and its interpretation can be utilized for (i) geological mapping, (ii) spotting of mineralized zones, and (iii) unravelling of structural elements like fault/shear zones, dyke swarms, and their alignments and extensions, boundaries of basinal and domal features. Aeromagnetic data can also be used to identify strike slip movements, rotational patterns, etc. and help in deriving basement configurations, thereby in the understanding of the structural evolution of different crustal domains. Here, we present a structural and tectonic scenario, derived from the interpretation of a small portion of the aeromagnetic data to look for terrains suggesting block rotation.

The study area forms a small portion of the granite greenstone terrain in Eastern Dharwar craton situated to the west of the Proterozoic Cuddapah basin. This area is bounded by 14°25′0″ and 14°35′0″N lat., and 77°30′0″ and 77°55′0″E long. (Figure 1 *a*).

The Archaean granite–greenstone terrain west of the proterozoic Cuddapah basin is a low to medium grade terrain, consisting of steeply dipping green schist belts, often invaded by highly elongated ‘granitoid bodies’. The terrain is traversed by NW, NE and ENE trending Precambrian dykes[18]. Dykes of younger ages traversing in other directions are also there. Quartz reefs are mostly emplaced along NW trending shear zones in this part of the area. A comprehensive geological history of this area is provided by Raju *et al.*[19]. The aeromagnetic lineaments in this area mostly represent dyke systems and fault domains. Two NW–SE trending parallel fault/shear zones, viz. the Hampapuram and Kandukuru faults (Figure 1 *b*) are cut by ENE trending faults, often occupied by ENE trending dykes. Quartz reefs are emplaced in both the fault/shear zones. Cataclastic and brecciated rock matrix are present in these two fault/shear zones. Displacement of dykes indicates a possible right lateral movement along the faults[9]. The aeromagnetic data for this belt has been reprocessed to infer structures, if any, related to the faults/shears and to bring out the tectonic history *vis-à-vis* the proposed block rotation hypothesis.

The National Geophysical Research Institute (NGRI) in collaboration with the Geological Survey of India (GSI) carried out an airborne magnetic survey for mineral exploration during 1980–82 over a part of the Eastern Dharwar craton and part of the Proterozoic Cuddapah basin, at a flight height of 150 m with a flight line spacing of 0.5 km in some selected areas and with a line spacing of 1 km in most of the area. The area under study (Figure 1 *a*) forms a small part of the aeromagnetic map on the Archaean basement complex.

The total intensity aeromagnetic anomaly field data of the study area have been reprocessed, and high-pass and low-pass filtered anomaly maps have been prepared using the MAGMAP software of Paterson, Grant and Watson Ltd., Canada. Reduced to equator (RTE) option has been applied on the above high-pass and low-pass filtered maps. These are shown in Figures 2 *a* and 3 *a* respectively. This option has been preferred because: (i) the high-pass filtering accentuates the shallow magnetic features at the expense of the broader and deep-seated sources, and allows one to identify easily the various shallow magnetic trends, (ii) the centres of the magnetic lows and/or highs in the RTE map clearly locate the tops of the sources. The low-pass filtered RTE map has been analysed to identify the possible downward continuity of the shallow structural features.

Aeromagnetic interpretation helps in recognizing long linear features like faults/shears, dykes and other structural lineaments. Offsets in continuity of the magnetic

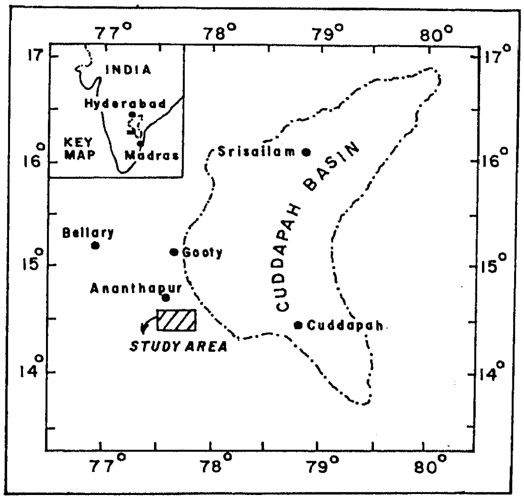

Figure 1 *a*. Location map of the study area.

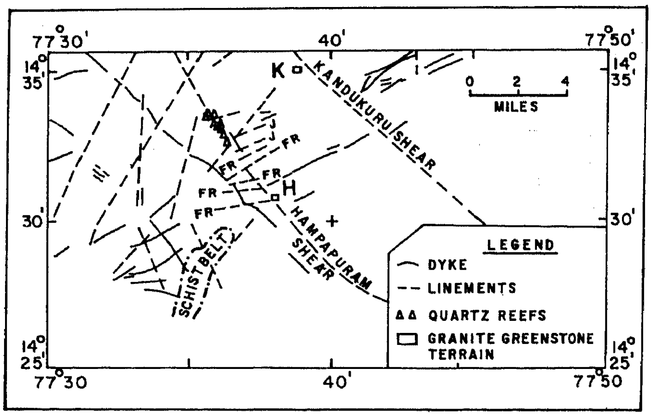

Figure 1 *b*. Geological map of the study area.

trends/lineaments, abrupt terminations of magnetic lows and/or highs, drag features, contour nosings, alignments of lows or highs, etc. are some of the direct indicators of fault/shear zones[20]. A persistent offset in the magnetic trends and drag features on either side of a fault/shear zone indicates the possibility of strike slip environment, even though large scale strike slip faults do not leave any morphological evidence for strike slip faulting.

The low geomagnetic latitudes such as the present area, an intensely sheared tectonic zone with a possibility of depletion and/or destruction of magnetic minerals occurring, can be identified by the characteristic positive magnetic anomaly closures all along the shear zone.

Using the above criteria, the aeromagnetic anomaly map of this area has been interpreted and lineaments, dislocation planes, possible shear/faults, etc. identified. The interpreted structures are shown in Figures 2 *b* and 3 *b*.

Two major structural elements that are dominant and striking on the map are the NW–SE trending Hampapuram fault/shear in the west and the Kandukuru fault/shear in the east. A number of ENE and EW trending magnetic lineaments, mostly representing fault/shear zones, often occupied by dykes, are also seen cutting across the NW features, thus forming a domain of fault system. The NNE–SSW trending magnetic anomaly feature represents the extended portion of the Hagari–Ramgiri schist belt. A general observation is that there are fault zones both in the north and south bounding the Hampapuram and the Kandukuru fault/shear zones. The ENE and EW features are found to have been offset by the NW faults. The extent of offsets are shown as d1, d2, d3, . . . in Figure 2 *b*.

Shifts in the NE and ENE trending magnetic anomaly

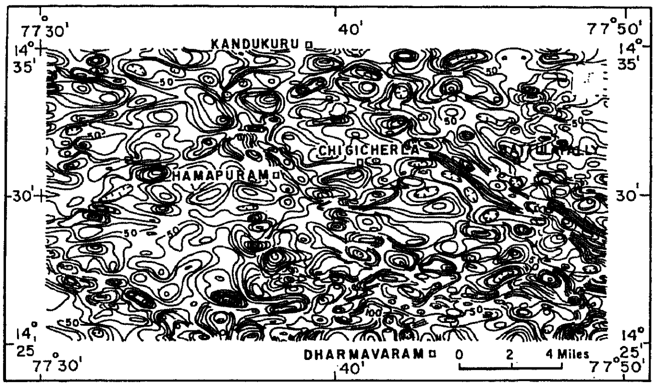

Figure 2 *a*. High-pass filtered, reduced to equator total intensity aeromagnetic anomaly map of the area (cont. interval 10 nT).

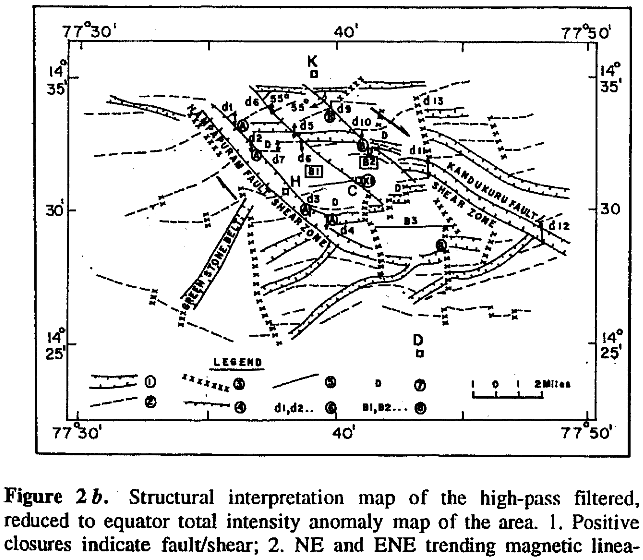

Figure 2 *b*. Structural interpretation map of the high-pass filtered, reduced to equator total intensity anomaly map of the area. 1. Positive closures indicate fault/shear; 2. NE and ENE trending magnetic lineaments; 3. Dislocation planes; 4. Possible fault traces within the block; 5. Inferred extensional fractures; 6. Extent of displacements along shears; 7. Drag features indicate strike slip movement; 8. Inferred rigid crustal blocks bounded by shears.

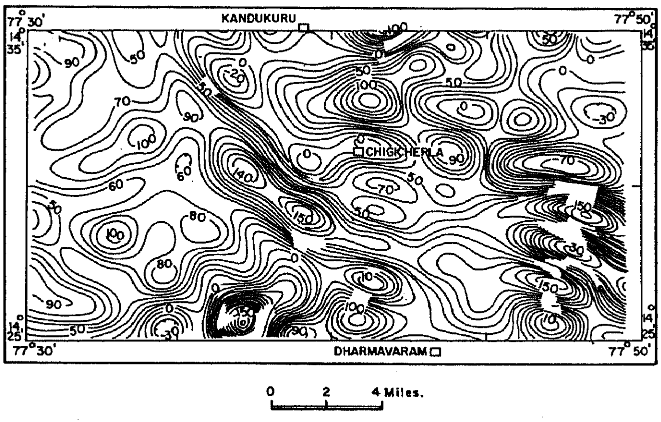

Figure 3 *a*. Low-pass filtered, reduced to equator total intensity aeromagnetic anomaly map.

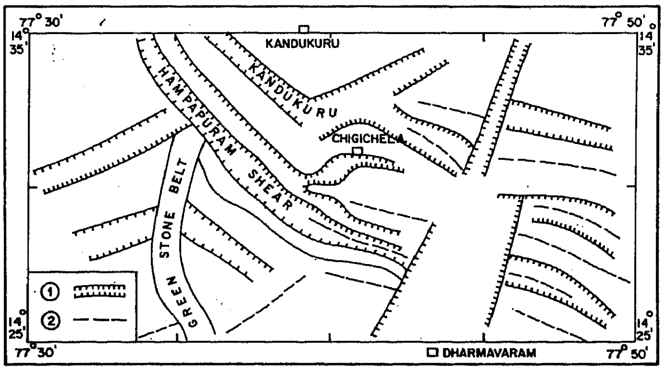

Figure 3 *b*. Structural interpretation map of the low-pass filtered, reduced to equator total intensity aeromagnetic anomaly map of the area. Note the existence of both the Hampapuram and Kandukuru fault/shear even at deeper levels.

trends (Figure 2 *b*), designated as 'A' on either side of the NW–SE trending Hampapuram fault and 'B' on either side of the Kandukuru shear zone and the drag features 'D', indicate strike slip movement. These shifts in the anomalies can be attributed to a right lateral movement along the shear zones. Field and other evidences also confirm right lateral movement in regional scale[9]. The positive magnetic anomaly closures as shown in the figure, are associated with intense shearing. Continued tectonic activity in this part of the craton has resulted in a sygmoidal shearing as well as rotational features in the faults (as indicated by arrows in the Figure 2 *b*). Thus, interpretation of this map led us to identify rigid crustal blocks B1, B2 and B3 bounded on all sides by strike slip faults which probably caused the blocks to rotate. Occurrence of kimberlite intrusion at one of the corners in the blocks B1 (shown as k1) seems to have been owing to a possible extension due to rotation in this corner. A clockwise rotation of the blocks owing to the strike slip is envisaged in this part.

Figure 3 *b* is the structural interpretation map of the low-pass filtered, reduced to equator data. This interpretation further supports the continuation of the two shear zones vertically downwards. The log normalized radially averaged power spectrum (Figure 4 *a*) computed for the low-pass filtered map gives an average depth of 3.0 km for a possible magnetic marker horizon. This depth, perhaps, represents the depth to a mid-crustal disharmony beneath the strike slip fault system[21]. Thus, geophysical inferences suggest a region of block rotation in which the blocks are surrounded by deformed rock matrix. Based on these structural inferences, a block diagram showing the possible strike-slip and block rotation in this region is presented in Figure 4 *b*.

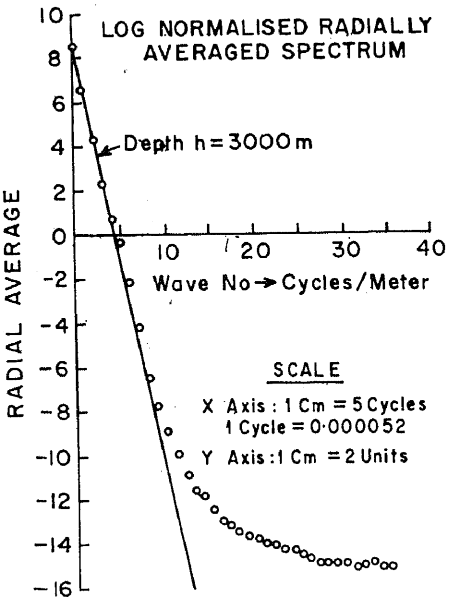

Figure 4 ***a*****.** Log-normalized radially averaged power spectrum computed for the low-pass filtered aeromagnetic anomaly field data. $h = 3$ km. Possibly indicates the depth to the mid-crustal disharmony beneath the shears.

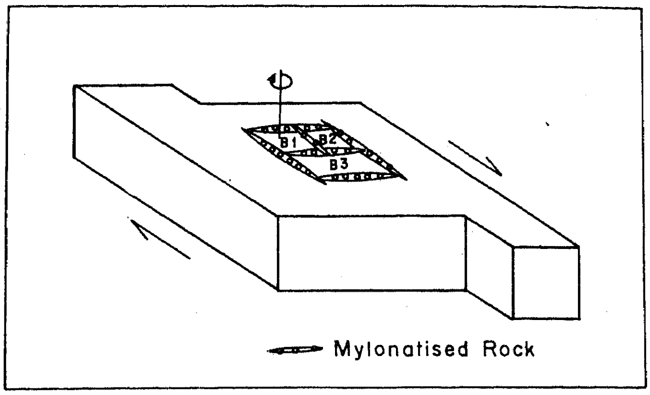

Figure 4 ***b*****.** Interpreted block diagram showing the inferred crustal blocks B1, B2 and B3 surrounded by the cataclastic and brecciated rock matrix.

An attempt is made here to calculate the angle of block rotation using the relation given by Nur *et al.*[22]

$$d = W \sin\phi\, [\sin\alpha \times \sin(\alpha - \phi)]^{-1}, \qquad (1)$$

where d is the displacement along the fault, W the width of the faulted block, α the initial angle between the faults and the boundary of the domain and ϕ the block rotation angle.

The displacement d is measured from the interpreted section. The average of the horizontal displacements on either side of each fault (here the displacements $d1$, $d2$, etc. between A's B's on either side of the faults) represents the strike-slip displacement along the faults.

The initial angle is the angle between the fault and the boundary as shown in the figure, and the width w is the average distance between the two faults.

Here $d = 680$ m (average displacement), $w = 3165$ m (Block 1), $w = 2850$ m (Block 2).

Substituting these values from the interpreted section, the angle ϕ is calculated from equation (1) and is 9.0 and 8.0 degrees clockwise for blocks 1 and 2 respectively.

Interpretation of a part of aeromagnetic data from a region of strike-slip movement in a Precambrian granite greenstone terrain, has resulted in identifying a zone of possible block rotation. The average angle of block rotation is determined from the width w, initial angle α and the displacement d measured from the interpreted map, using the relation given by Nur *et al.*[22] and is 8.5 degrees clockwise. Computation of the radial power spectrum from the low-pass filtered aeromagnetic anomaly data of this area, has given an average depth to a possible magnetic marker horizon at a depth of 3.0 km. This probably represents the depth to the decoupling zone in the upper crustal level below the shear zone.

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Statistical analysis of glaciers in Himachal Pradesh, north-west Himalaya, India

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A statistical analysis of Himachal glaciers has been carried out. The maximum concentration of glaciers is in the elevation range 4500–5500 m. More glaciers are of transverse simple type than longitudinal type.

THE Himalaya, on the southern slopes of the Tibetan plateau, is one of the most important glacier systems on the earth. The main snow-gathering ground is in the higher Greater Himalayan ranges, whose relationship with climate changes on various time scales indicates topographic and climatic conditions favourable for the formation of the glaciers. The present work is in continuation of our glacier inventory[1].

The study area is situated in the western part of the Himalaya, extending about 315 km between UP Himalaya in the east and the Kashmir Himalaya in the west (Figure 1). It encompasses mountain area of about 48,034 km^2 out of the total land area of 55,673 km^2 of the Himachal Himalaya. It is estimated that 9.4% (approximate 10%) is glaciated area of 4516.22 km^2 out of the total mountainous area of 48,034 km^2. Every 1 km^2 of the land area is glaciated to the extent of 0.09 km^2. Six hundred one glaciers have been identified in six drainage basins[2], Chandra–Bhaga (200), Beas–Parbati (80), Ravi (54), Malung (40), Spiti (159) and Satluj (68). Statistical analysis of glaciers in these drainage basins has been carried out on the basis of earlier works[2–8].

Glaciers are unevenly distributed between the Great Himalayan, Pir-Panjal and Dhauladhar ranges, controlled by the topographic variation and the amount of precipitation in the region. The altitude increases from NW to SE, where maximum precipitation occurs. So, the Satluj basin, which receives more precipitation and is at higher elevation, remains covered with snow for longer duration than the other basins. In this study the identification of glaciers is from LANDSAT imagery. Data for the glaciers are summarized in Tables 1 and 2.

In Chandra–Bhaga, Spiti and Satluj basins the highest frequency of glaciers is in the length range 2–4 km, while in Beas–Parbati, Ravi and Malung basins the highest frequency is around 2 km (Figure 2 *a*). It is also observed that 35% of the glaciers fall in the range 2–4 km in length, 55% are around 5 km, and the remaining 10% are 10 km or more in length. Bara-Shigri is the longest glacier (29 km) of the Himachal Himalaya.

The area covered by individual glaciers was calculated by digital planimeter. Figure 2 *b* shows that majority of the glacial cover area of Himachal extends over 2 to 6 km^2, but some glaciers are more than 10 km^2 in area. An examination of Table 1 shows that the Chandra–Bhaga, Malung and Spiti basins are relatively balanced in basin area and glaciated area, while the Beas, Ravi and Satluj are less glaciated compared to the basin area. The glaciers in NW Himachal Pradesh are bigger than those in the SE region.

Broadly the glaciers have been classified into two main types (i) Longitudinal and (ii) Transverse, on the basis of their flow direction[3]. These are further subdivided into simple and compound types. Simple glaciers have a single accumulation zone while compound glaciers have two or more accumulation zones in a single basin.

About 69.5% of the Himachal glaciers are of transverse simple (TS) and compound (TC) types and 30% are of longitudinal simple (LS) and compound (LC) types. The largest class of glaciers is the transverse simple (TS) type (Figure 3) while longitudinal glaciers are fewer, and occupy wider valleys and are larger in size. The distribution of transverse and longitudinal glaciers in different basins is, respectively:

Chandra–Bhaga,	67 and 33%;
Beas–Parbati,	74 and 26%;
Ravi,	58 and 42%;
Spiti,	65 and 35%, and
Satluj,	75 and 25%.

Glaciers in Malung basin are of only transverse type. It is well known that the orientation (flow direction)

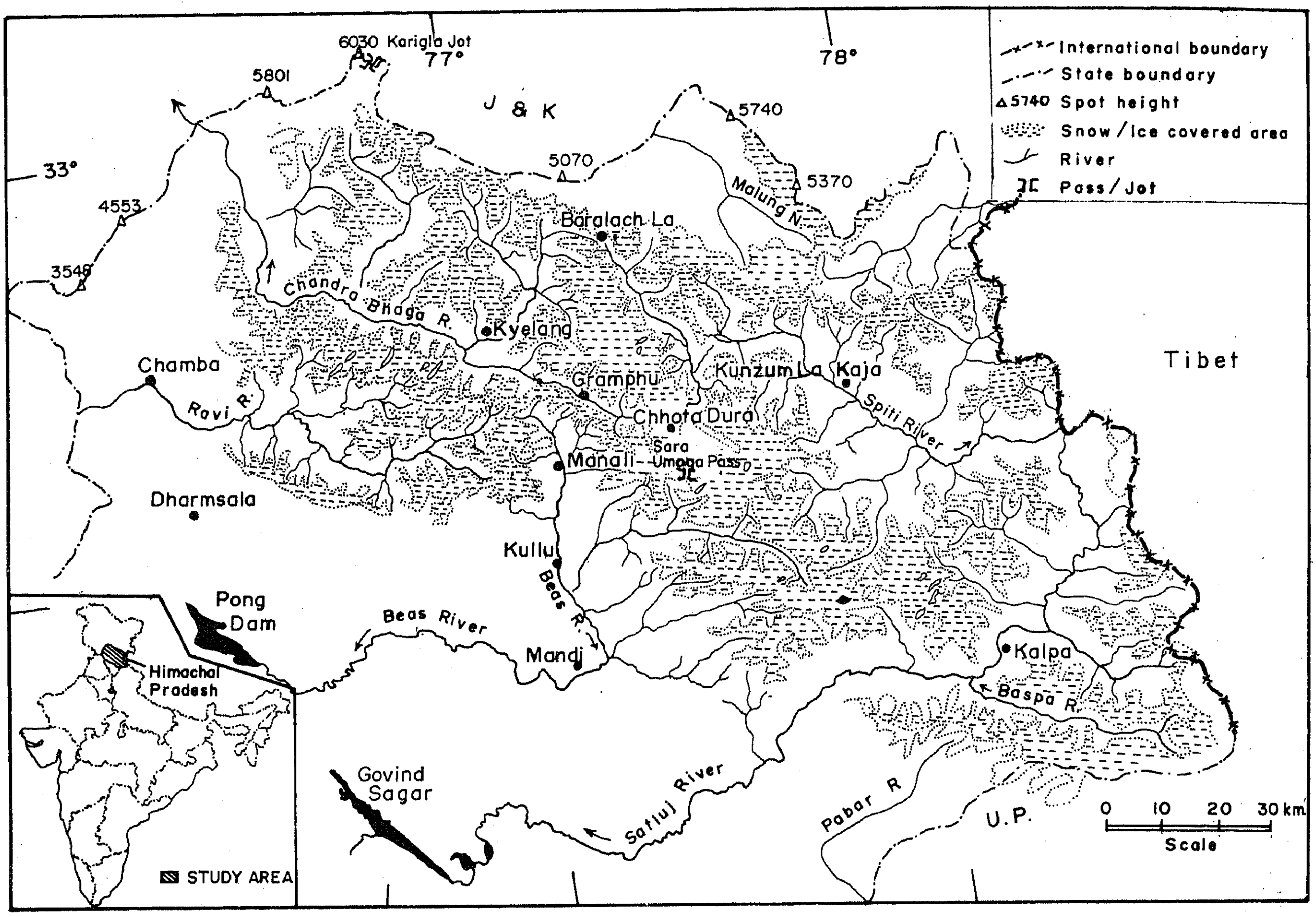

Figure 1. Permanent snow cover and glaciers regime map of study area (H.P.) (Based on Satellite Data, Oct.–Nov. 1988).

Table 1. Number of glaciers identified in different basins

Basin	Basin area (km^2)	Glaciated area (km^2)	No. of glaciers	Average size (km^2)	Ratio between basin and glaciated areas
Chandra–Bhaga	7510	1778.98	200	8.89	4.22 : 1
Beas–Parbati	14,070	729.59	80	8.03	19.28 : 1
Ravi	5154	340.97	54	6.03	15.10 : 1
Malung	1570	231.86	40	5.78	6.77 : 1
Spiti	5440	1151.12	159	7.23	4.30 : 1
Satluj	14,290	283.70	68	4.17	50.00 : 1
Total	48,034	4516.22	601		

Table 2. Summarized statistics for the glaciers in six basins

Parameters	Chandra–Bhaga	Beas–Parbati	Ravi	Mulang	Spiti	Satluj
Total basin area (km^2)	7510	14,070	5154	1570	5440	4290
Glacierized area (km^2)	1778.98	729.59	340.97	231.86	1151.12	283.70
Glacierized area % of basin area	23.60	5.19	6.62	14.77	21.16	1.99
No. of glaciers	200	80	54	40	159	68
Length						
a) >5 km	40	16	07	07	15	03
b) <5 km	160	64	47	33	144	65
Types						
Longitudinal	66	21	23	–	56	17
Transverse	134	59	31	40	103	51
Largest glacier						
Length (km)	29	17.50	9.00	5.5	10.00	6.50
Area (km^2)	157	82.00	18.75	13.8	26.63	15.54
Elevation						
Maximum (m)	6200	5500	5400	6000	6000	6000
Minimum (m)	4000	4000	4000	5000	5000	5000

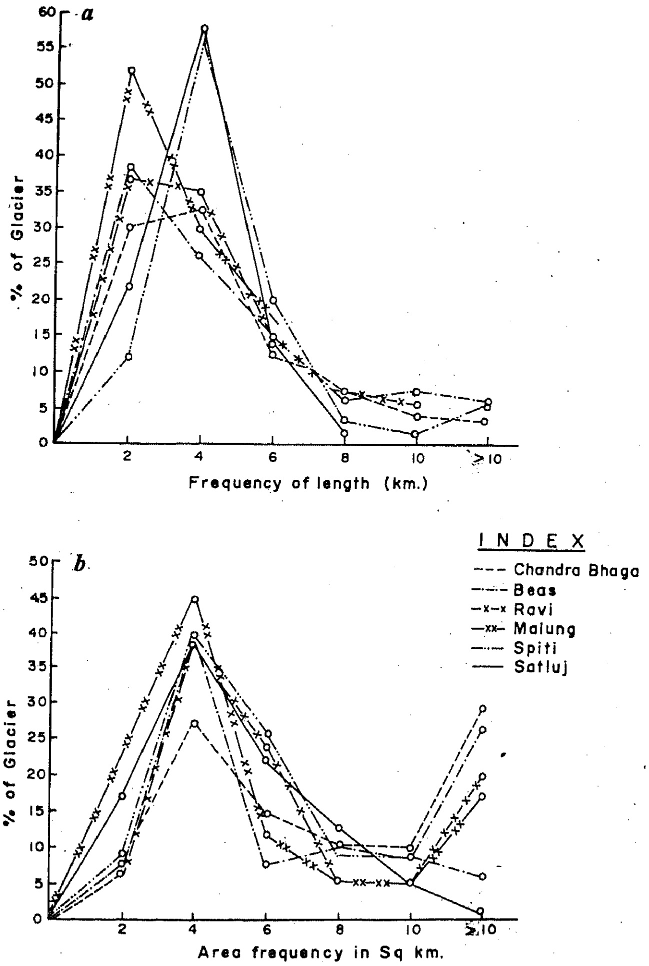

Figure 2. Frequency diagram of (*a*) length vs glacier % and (*b*) area vs glacier %.

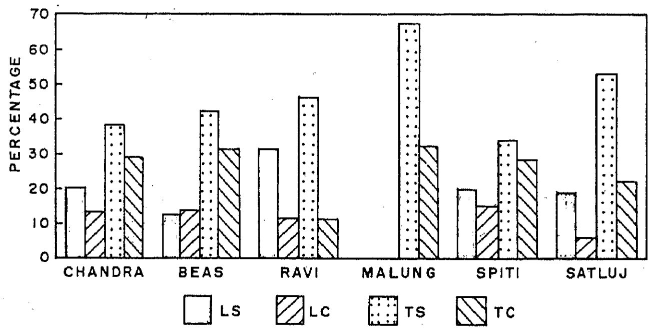

Figure 3. Distribution of glacier types in different basins.

of the glacier is controlled by topographical slope of the region. Most of the Himalayan glaciers are oriented N–S whereas valleys are mostly E–W or parallel to the orographic trend of the Himalaya. In Himachal Pradesh the orientations of identified glaciers have been determined and rose diagrams prepared on the proportion of glacier catchment in sixteen compass sectors (Figure 4). Glaciers in Chandra–Bhaga, Spiti, Malung and Satluj are mostly northward oriented (> 50%), while the majority of glaciers in Beas–Parbati and Ravi basins are southward. It is also observed that glaciers with northward orientation are longer and cover larger area, for example Bara Shigri (29 km covering 157 km^2), Mujrilangang (13.75 km covering 30.7 km^2) and Chhota Shigri (9 km, covering 8.75 km^2); southward glaciers are smaller in length, cover less area and are steeper.

The maximum concentration of the glaciers is between 4500 m and 5000 m altitude, while a few of them extend down to 3800 m. The lower limit (snout position) of

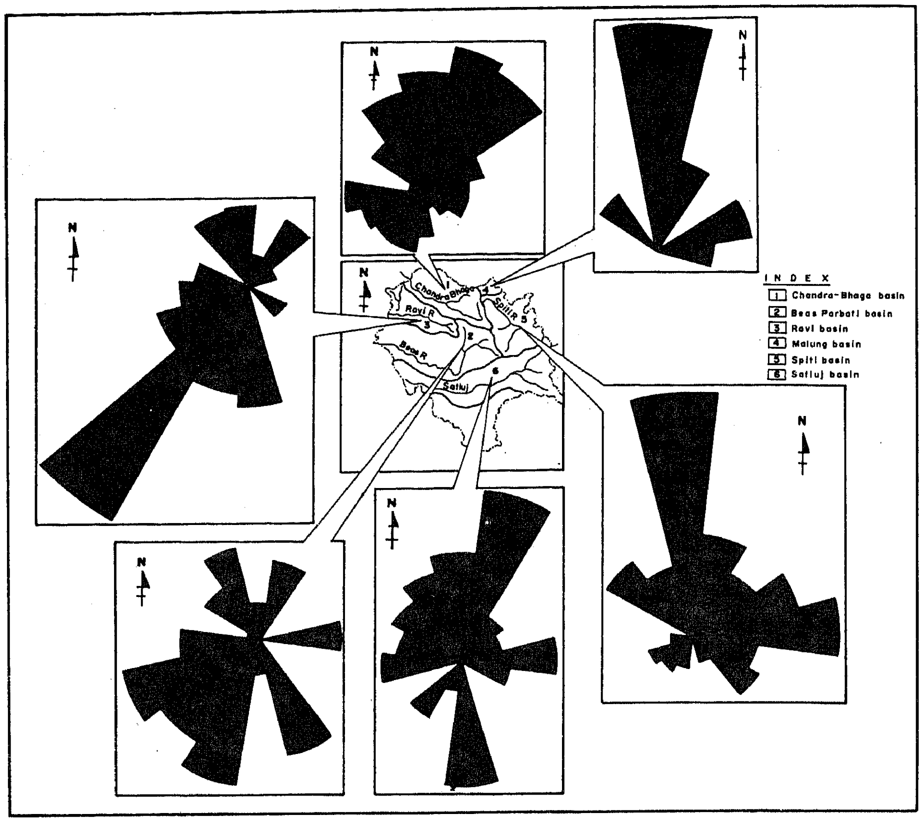

Figure 4. Glacier orientation in different basins of Himachal Pradesh.

the glacier fluctuates between 4000 and 5000 m in different basins. The orientation of the glaciers depends on the topographic conditions and the orographic control of the mountain. Thus orientation is spread over in all directions, with main concentration northward (58%) and southward (32%).

The slope/gradient of the glaciers varies between 3 and 33 degrees, but sometimes sudden increase in slope gives the evidence of surging or ice cliff on the glacier body. Where the slope is more the snow does not stand longer. Such glaciers can be considered as hanging glaciers and become vulnerable to avalanches and other glacier hazards.

Glacier development in a terrain is dependent on the topographic and climatic conditions of the region. Study of glacier development is useful for understanding present and past climatic and environmental changes. Statistical comparison of higher latitude glaciers with lower latitude glaciers (Himalayan type) can give an idea and correlation of control of precipitation and palaeoenvironment.

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Knot Theory and its Applications. Kunio Murasugi. Birkhäuser Verlag AG, Klasterberg 23, PO Box 133, CH–4010, Basel, Switzerland. 1996. Pages 323. SFr 98, DM 118.

Even though mankind has been using knots ever since the dawn of civilization, the so-called theory of knots which came into existence as a special branch of three-dimensional topology is approximately only a hundred years old. It had some reasonable initial development followed by a slow growth for a few years. And then, with its multifarious applicability in other sciences, it has grown so much and also growing rapidly that today it can be treated as a separate branch of Mathematics in its own right.

All these days, anyone who wanted to know what knot theory is always started with the excellent, 1962 survey article 'A quick trip through knot theory' by R. H. Fox or with the book *Introduction to Knot Theory* by Crowell and Fox. However, even these two expositions demanded quite a bit of preparation in algebraic topology and indulgence from the reader. For instance, the knowledge of fundamental group and the presentation of groups was very essential. Not so any more. The present volume by KM is a proof that anybody can acquire a decent knowledge of a lot of useful knot theory without much expertise in algebraic topology. Today, if somebody approached me for some elementary knowledge of knot theory, I can place KM's book in her/his hands without any hesitation.

Mathematically speaking a knot is merely a smooth embedding of a circle in the 3-dimensional Euclidean space. One can project the entire knot onto a plane to obtain a smooth curve with only finitely many points of overlaps. We can also arrange this projection in such a way that at each of these overlaps, only two of the strands meet and they are perpendicular to each other. By making a small cut while drawing one of the two strands at the meeting point, we indicate which one lies underneath the other. Such a projected plane figure is called a regular knot projection.

The entire knot theory can be carried out from studying such a regular knot projection. Thus, many ideas and concepts about knot theory can be understood by merely drawing appropriate plane figure. Since we are not interested in the actual length of the knot etc., it is fairly obvious that we can introduce a number of operations on the knot which alter it considerably and yet treat it as 'equivalent' to the given knot. These operations are called elementary knot moves.

How far one can go on like this? KM could come up with a whole neat volume written in this style without much technicalities. He declares his intentions of keeping the book totally devoid of all clumsy technicalities and he has succeeded in it. The equivalence of knots, sums of knots and prime knots, knot-cobordism and slice knots, what it means to construct knot tables, and the fundamental problems of knot and link theory are explained in the first three chapters. The classical knot invariants such as the minimum number of crossings, the bridge number, unknotting number, the linking number, colouring number, etc. occupy the next chapter. It all looks so soft so far. However, already, the author points out many open problems which have defied solutions for all these days.

In chapter 5, the theory comes to one of the major turning points in its history. The Alexander polynomial has played a very important role in the classification of knots. Classically, one introduces this concept via the so-called free differential calculus of the presentation of the knot-group or through covering spaces. This approach is quite technical and not surprisingly, KM avoids it and introduces the Alexander polynomial through the Seifert matrix. Thus chapters five and six deal with Seifert surfaces, s-equivalence and Alexander–Conway polynomial, signature of a knot and its usefulness in detecting chirality of knots. The author does not forget to point out the efficiency of Conway's method in making the Alexander polynomial more computer-friendly. It is remarked that this is a case of technology catching up with the mathematical theory.

Chapter 7 contains a satisfactory classification of those knots which can be drawn on the surface of a standard torus.

In chapter 9, the author comes up with another important step in the development of knot theory, again due to Conway, viz. the theory of tangles. The next logical step is the study of braids and the relation with the knots and links. An important theoretical result here is Markov's theorem. It says how two different braids arising from the same knot are related, viz. by the so-called M-equivalence.

We are naturally led towards the great revolution – the Jones Polynomial, serendipitously discovered by V. F. Jones in 1984. An important aspect of the Jones polynomial and its subsequent offshoots is in the fact that they have unlocked connection of knot theory with various applicable disciplines. Of course, it is not necessary to go through the same route as Jones took via the study of operator algebras to understand Jones polynomial. One has to merely axiomatize the skein relations and that is what is done in chapter 11. After discussing the basic properties of the Jones polynomial, KM already includes open problems. For example, it is not known whether there exists any non trivial knot with its Jones polynomial equal to 1. The chapter includes discussion of some of the other offshoots such as the HOMFLY polynomial, the Kauffman's polynomial, etc. It concludes with proofs of three of Tait's conjectures about alternating knots.

The next three chapters treat relation of knot theory in three applicable disciplines – the statistical mechanics, molecular biology and chemistry. These chapters are useful to people working in either areas.

The last chapter treats the most modern concept in knot theory – Vasileiv invariants. Indeed, these invariants are assigned to even a wider class of objects, viz. singular knots and as such they are more powerful than all the invariants considered so far. However, it is not known whether Vassileiv invariants form a complete set of invariants for a knot. Examples of knots not distinguishable by any given finite set of Vassileiv invariants are included.

The book contains two tables, one giving knots up to eight crossings and the other containing Alexander and Jones polynomials for these knots. Notes containing eighteen footnotes is included. As the author points out the bibliography is strictly for the work referred in the body of the book, other references on the subject can be obtained from these sources in turn.

It goes without saying that, one could

not have given proofs of many results, with such a self-imposed restriction of avoiding technical details. Nevertheless, the author has done a very good job, the only exception being chapter 8, in which the author attempts to outline aspects which are more topological in nature such as the Dehn's surgery, branched coverings, etc. A typical exercise in this chapter asks the reader to show that the torus is not a covering space of a 2-dimensional sphere. One fails to understand the kind of audience the author may have in mind, in including such exercises immediately after giving the definition of a covering space. The book can do well without these eighteen pages. This book is translation of the original Japanese version. Even though I do not know Japanese, I feel that there is a lot of scope for an improvement in the translation.

Although this book may not impart any lasting education in knot theory to the reader, it will not fail to inspire and inform substantially. With so many results in one single place, it may be used as a good reference book also.

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Lysozymes: Model Enzymes in Biochemistry and Biology. P. Jolles, ed. Birkhäuser Verlag, Basel, Switzerland. 1996. 449 pages.

Fleming's discovery of lysozyme preceded his discovery of penicillin and both are part of scientific folklore. It was in 1922 that Alexander Fleming reported his discovery of lysozyme in the nasal mucous to the Royal Society. However, when the word lysozyme is currently used, it generally means the enzyme from hen egg white (HEW). Pierre Jolles, who sequenced the HEW enzyme in the early sixties, has been working with lysozymes from various sources and has now put together this comprehensive book on this enzymatic activity.

Lysozyme is widespread in nature. By definition, lysozyme activity consists of hydrolysis of a ß-glycosidic bond between the C-1 of *N*-acetylmuramic acid and the C-4 of *N*- acetylglucosamine of the bacterial peptidoglycan. Analysis of known 75 complete and 13 partial amino acid sequences shows that there are 20 invariant residues. In addition to conventional lysozyme (called lysozyme c for chicken type or conventional type), other distinct types of lysozymes also occur. Lysozyme g (after the Embden goose, the specie in whose egg white it was first discovered) is also widespread, though in bird egg white only. Some lysozyme c, notably from pigeon egg and horse milk have few aspartic acid residues critically positioned, enabling them to bind calcium and are called calcium-binding lysozyme c. The two lysozyme c families along with α-lactalbumin form a lysozyme c superfamily. Evolutionary analysis points to a common ancestor and places the divergence event prior to 400 million years. The question whether lysozyme g also shares the common ancestor is still debatable. However, v-type lysozymes (viral type from phage infecting both gram-positive and gram-negative bacteria), on the basis of similarities between their three-dimensional structures with lysozyme c, are believed to share this remote common ancestor.

Several other phage lysozymes encoded by phages infecting gram-positive bacteria called CH-type lysozyme (first investigated in Chaloropsis) belong to a totally unrelated family. A striking observation which emerges out of these evolutionary studies is that only acid catalysis seems to be essential, the rest of the catalytic process is based upon 'broadly scattered interactions (electrostatic, H-bonding, . . .) and substrate distortions' (p. 58).

The role of lysozyme in bacterial cell wall lysis was elucidated only in 1964. 'An intriguing question, not yet answered, is how the cell controls this dangerous enzyme from premature or unbalanced action. . . although it is clear that they are involved in the metabolism of bacterial cell wall' (p. 63). Equally fascinating is their presence in plants. 'All lysozymes also have chitinase activity but not all plant chitinases are lysozymes. However, for many chitinases, it is not known whether they also possess lysozyme activity' (p. 75). As fungal cell walls contain chitin, it is believed that chitinase activity is used by plants to combat fungal pathogen.

Lysozyme (c-type) is ubiquitous in insects, normally present in blood. In this context, the role of lysozyme as an active defence molecule has been questioned, since in most bacteria, the peptidoglycan layer is not directly accessible to this enzyme. It may be that the main role of the enzyme is in cell wall lysis after the bacterium has been killed.

Both in flies and cows, lysozyme also has a digestive role. Whereas typical lysozymes are basic, ruminant enzymes are neutral and Drosophila midgut lysozymes are even acidic.

Thus this book, consisting of twenty-two chapters, looks at lysozymes from the perspective of protein chemistry, enzymology, protein crystallography, molecular biology, immunology and pharmacological and therapeutic applications. The authors, drawn from various parts of the world, represent a unique wealth of experience.

An attractive feature of this book is that it also captures a sense of history of the growth of ideas. McKenzie's recall of editorial opposition to Campbell's opinion that 'α-lactalbumin may have evolved by gradual modification from lysozyme' is a case in point (p. 365). The lucid discussion on the catalytic mechanism by Karplus and Post nicely illustrates the usefulness of theoretical methods in establishing enzyme mechanism. As few biochemists are familiar with the area of molecular dynamics, the inclusion of this chapter is welcome.

Analysis of formation of disulfide bonds in this enzyme shows that there exists a restricted search of structures and a nucleation in the folding pathway. 'Folding of both denatured and denatured/reduced lysozyme is characterized by transient folding species possessing structural properties of the molten globule state: high content of secondary structure, no tertiary fold and appearance of hydrophobic structures' (p. 144). Imoto has described protein engineering work on c-type lysozyme (p. 163 onwards). Although references to the work on T4 lysozyme by Mathew's group have been provided, a little more extensive discussion on the latter would have been welcome. Also missing is the early chemical modification with the enzyme.

HEW lysozyme was the first enzyme

in which case the three-dimensional structure was available from X-ray crystallography. It is interesting to recall that X-ray crystallography of lysozyme gave the first view of ß-sheet in a globular protein. The X-ray diffraction studies on binding of substrate analogs, transition state mimics and oligosaccharide products to various lysozymes have provided considerable insight into binding of sugars to proteins.

Evolutionary variants of bird lysozyme played a major role in the development of correlation between immunological and sequence differences. 'The anti-hen egg lysozyme monoclonal ab DI.3 is perhaps the best characterized immunoglobulin to date' (p. 303). The chapter by Prager on adaptive evolution of the enzyme focuses on lysozyme c turning into a digestive enzyme and in a subsequent chapter, Irwin discusses this as a link between molecular evolution and evolution at the level of organisms.

The last chapter by Sava outlines the underexploited potential of therapeutic applications of lysozymes. However, a very important warning of general importance is given by Imoto (p. 177), 'Engineered proteins might be sometimes harmful to human bodies; only one amino acid substitution (Ile 55 Thr or Asp 66 His) turned human lysozyme to an amyloid fibril protein'.

In a way, this book also reflects the state of the art in the area of enzymes in general. Pierre Jolles has yet again made a tremendous contribution to biochemistry.

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Haridas Banerjee: A self-taught physicist

Introduction

The Indian Journal of Physics brought out a special felicitation volume, 1996, **A70**, No. 3, on the occasion of the 60th birthday of Professor Haridas Banerjee who retired from the Saha Institute of Nuclear Physics in May 1995, and joined the S.N. Bose National Centre for Basic Sciences as INSA senior scientist. This volume contains an accurate biographical sketch by Samir Mallik, a complete list of the 45 publications of Banerjee and a set of research articles in tribute.

When Ramaseshan requested me to write an article about Haridas Banerjee, the immediate problem was – what could I write that is not already in the felicitation volume? I decided to include his reminiscences told to me which might be of interest to the young student starting out in research. The material opportunities for research are decidedly better today due to the enormous increase in instantaneous communication of research results worldwide. On the other hand, the career opportunities for a research physicist in comparison with other emergent avenues are still such that some dedication to science is involved in making the choice. On balance, the human dimensions of the choice are not very different from what it was 40 years ago.

Why research?

Born on 1 May 1935 at Mymensingh, Bangladesh, Haridas Banerjee faced the choice in 1955 after an M Sc in Applied Mathematics from Calcutta University. Research was not on his list of priorities. He wanted to be an actuary (insurance) and his parents wanted him to be a civil servant (IAS). Things started changing as he came either under the spell or in the proximity of great teachers and scientists which included among others, men like Satyen Bose and Maghnad Saha. But he could not marshall enough courage to take the plunge in research. Research, he thought, could not be done on an empty stomach – one needed adequate financial cushion to get enough time for serious thinking. He had, before him the examples of some of his teachers, who even at a ripe old age were lecturers at Science College earning only Rs 250 p.m. So, in February 1956, when he was given a standing offer of a Senior Research Fellowship at IIT, Khargpur he did not accept it. Instead, he followed the wishes of his parents and started preparing for the competitive examination for IAS. In retrospect, he finds the wishes of his parents quite natural. Theirs was a refugee family uprooted from their homes in East Bengal in 1948. What better security could there be for his parents than having him installed as a Magistrate in some district?

Things changed rapidly in June 1956. First, he discovered that lecturers in IIT, Kharagpur had the same salary scale as that of a beginner IAS bureaucrat. So, he was assured that one need not do research on an 'empty stomach'. Second, his old friend and room-mate in the Hindu Hostel of Presidency College, Sukhomoy Chakraborty, who was doing research in economics at ISI, Calcutta, virtually chastized him. His words were 'What nonsense: why should you care to become a civil servant in independent India?'

Thus, in August 1956 Haridas landed in IIT, Karagpur to claim the Senior Research Fellowship offered six months earlier. For his parents this was a setback, but he was determined.

The challenge

It now appears truly remarkable that Haridas, without any training in theoretical physics was never daunted by the fact that, leave alone quantum field theory, even quantum mechanics was for him a totally unknown territory. He just took the plunge. Perhaps, the shining examples of the illustrious alumni Saha and Bose were still fresh in his mind. The assurance of some of his teachers that he should be quite capable of taking up on his own any new area in theoretical physics, provided the moral support.

Early successes

It took him about a year to teach himself basic quantum mechanics and then quantum field theory. The book *Theory of Photons and Electrons* by Jauch and Rohrlich was just out and he found it extremely helpful. By August 1957 he was ready to embark on research proper. Parity violation in weak interactions had just been discovered and experimentalists were eager to measure the polarization of beta particles. For this they badly needed the formulae for polarized cross-sections for processes involving electrons and positrons. In quick succession of a series of papers Haridas presented the required formulae for Bremsstrahlung, Mott scattering and photo-electric effect. His paper on Bremsstrahlung and that of Christian Fronsdal and H. Uberall of UCLA with identical results appeared simultaneously in *Physical Review*. Ugo Fano and his collaborator sent a paper on polarization effects in photo-electric phenomenon for publication in *Physical Review* without realizing that his paper on the same subject had been published in *Nuovo Cimento* about six months earlier. Their paper finally appeared after Fano incorporated extensive modifications on the basis of feedbacks from Haridas. By the time Haridas submitted his Ph D thesis in June 1959, his papers were well known among workers in the subject, both experimental and theoretical.

Jauch, the examiner of his thesis invited him straightaway to join the Physics Department of Geneva University. Thus, even before he was awarded the Ph D degree in December 1959, he was appointed Chef-de-Travaux in theoretical physics, a tenured position in Geneva University with effect from October, 1960.

The two great influences

During the earliest spell (1960–61) of his stay in Geneva he came in contact with two great physicists Josef Maria Jauch and Leon Van Hove, whose life and work had an abiding influence in shaping his attitude to research. Jauch used to tell him that our works were like our children and we should avoid doing something which might lead to embarrassment. Haridas' first encounter with Van Hove was rather accidental. Accompanied by Jacques Prentki, Van Hove came to his seminar (early 1961) on the Nambu-Jona Lasinio paper on spontaneous breaking of chiral symmetry. At the end of the seminar Van Hove came up to Haridas to offer his congratulations and appreciation. From Van Hove, Haridas imbibed the lesson that in research in theoretical physics, experiments are the elixir of life. A theoretical work is lifeless if it cannot be related to experiments.

Research at home vs research abroad and independence

Haridas never harboured – at least in the early years of his research career – the idea of settling abroad. But before returning to India, he wanted to visit the US. Thus, at the end of the first year at Geneva he told Jauch about his intention to resign. Jauch lamented 'You are leaving me like a hot potato'. Later on when Haridas visited Geneva again in 1969, this time with an one-year assignment at CERN, Jauch told him that had he stayed on in Geneva University, he would have become a Professor there. Haridas went to the US with an assignment at Carnegie Institute of Technology, Pittsburgh in October, 1961 and returned finally to India in October, 1963 to join Delhi University as a Reader in the Physics Department. Half-way during his stay at Carnegie Tech he made a short visit to India to get married to Sunanda, a charming, accomplished woman. From Delhi he moved on to Calcutta in April, 1966 to join Saha Institute of Nuclear Physics where he worked till his retirement in May, 1995.

Apart from socio-cultural alienation, there is something else that dissuaded Haridas from settling abroad. It has been his perception that in important centres like CERN, there is an under current of pressure to follow a set agenda in research. Conformism was at a premium, deviation had a penalty. Haridas however, enjoyed a lay-back style of functioning in research where priorities are set by himself. To quote his student Samir Mallik, Haridas prefers working with others but on problems formulated by himself. Indeed, Haridas is fiercely independent to a fault: he is not a good listener.

Loss of Sunanda and its impact

The loss of his wife Sunanda in March, 1984 was a devastating experience. 'It was as if I lost one leg and to support myself spiritually I had to depend entirely on the remaining leg, my research. My reflexes and attitude were that of an one-legged disabled person, jumping from one challenge to the next in a constant quest of excitement to fill the spiritual vacuum.' He was prepared to take more risks and address challenging and 'controversial' problems. In his own words, 'my children' of this period will continue to be a source of joy and happiness for years to come.

The present

Indeed, Haridas continues to be actively involved just now in the problem of putting fermions on the lattice which, for him, is an entirely new track. As a Fellow of INSA, he takes sincere interest in the activities of INSA and Indian science in general; his particular desire is that more research is done at the Indian Universities. I have known Haridas to be a simple, sincere, serious and hard-working physicist. He is always bubbling with enthusiasm that is infectious and has produced 10 Ph Ds. He is a fiercely independent physicist who has blazed his own track, always treating basic problems which inspire him, independent of what the prevailing fashions are. The canvass of problems on which he worked is truly wide: polarization effects in quantum electrodynamics, dispersion theory sum rules for hadron-scattering, multiparticle production in electron-positron scattering, the U(1) and CP problems in modern gauge theory. Concerning the last cited problem, Fujikawa remarked in the felicitation volume for Haridas Banerjee: 'H. Banerjee and his collaborators continuously clarified the most fundamental aspects of the problem and suggested a careful reassessment of the path integration quantization of modern gauge theory itself.'

S. M. Roy

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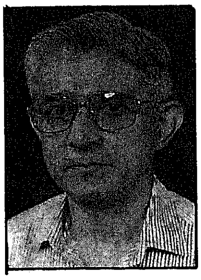
The Indian Academy of Sciences elects new Fellows – 1996



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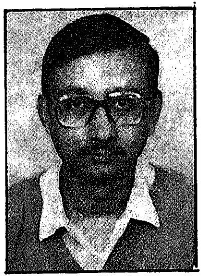

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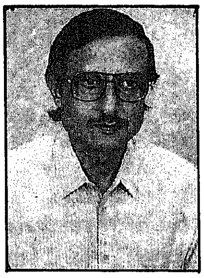

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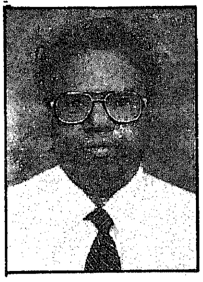

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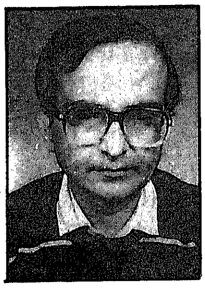

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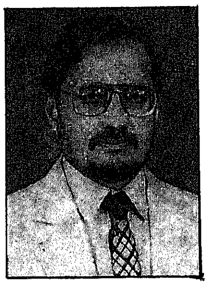

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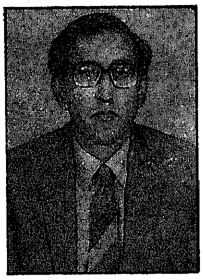

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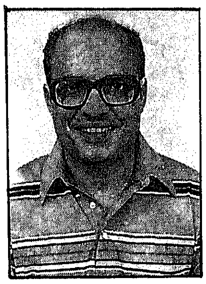

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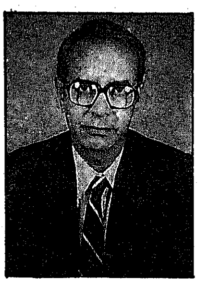

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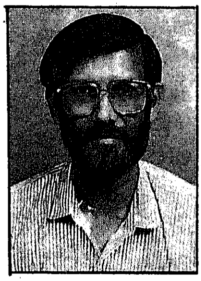

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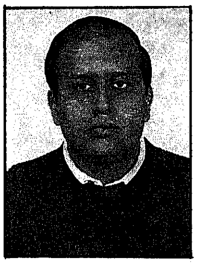

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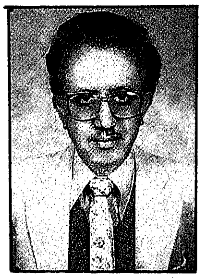

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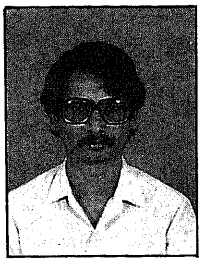

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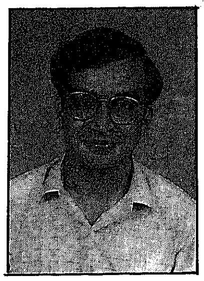

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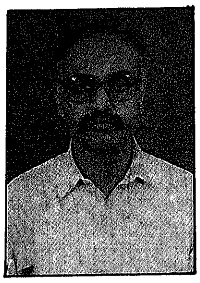

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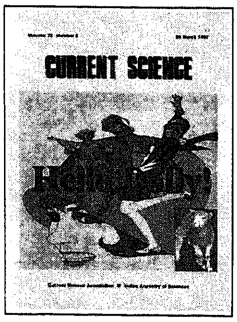

COVER. How far away are we from the days of human clones? See page 362.

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Sister chromatid cohesion during meiosis

Life is tough. To live, cells must not only divide to multiply but must do so correctly, again and again. The 'cell cycle' has been an area of intense recent study. Much of these studies have been done in the yeasts *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*, in vertebrate cells cultured in the laboratory and in the fruit fly *Drosophila melanogaster*. These studies bear out what is now a cliché worth repeating: The impressive evolutionary conservation of molecular players performing similar cellular functions. These studies on cell cycle and similar studies on the mechanisms of DNA and chromosome replication during the cell cycle have had immense practical relevance to medical problems. After cell division, each daughter cell must receive the correct complement of its DNA, present in the correct manner in each of its several chromosomes. To do this, the mother cell replicates each chromosome and the 'sister chromatids', the replicated chromosomes, 'stick' together to their homologs. At an appropriate time during the cell cycle, the 'sister chromatids' separate and one copy of each chromosome goes to each progeny cell.

The cell cycle can be conveniently divided into four stages. DNA replicates during the S phase and the duplicated chromosomes separate during the M phase. In the cells of our soma, as distinct from germ cells that give rise to egg or sperm, the S and M phases are separated by what are called gap or G phases. The G_1 phase occurs before DNA replication and the G_2 phase before mitosis or cell division. The progress of the cell cycle is subject to controls. There are 'internal' controls or check-points that co-ordinate cell cycle events in space and time and halt progression of the cycle in situations where irregularities occur. These check points will detect, for example, improper chromosome spindle assembly or DNA damage. Another type of control on the cell cycle are 'external controls'. The controls, which can be hormonal, controlled by growth factors or, for example, by neuronal signals; act to determine the extent and time of cell proliferation and have the effect on deciding the time of differentiation, the number of cells that will differentiate and where they will differentiate. In a multicellular organism, therefore, these controls on cell cycle are crucial in deciding the growth and form of an animal or plant. Integrating studies on cell cycle with those on the development of organisms is another exciting area of current research.

Coming back to 'sister chromatid' cohesion and separation, this aspect of the cell cycle has been studied in situations where many of the controls mentioned above fail to operate and in other kinds of situations as well: the timing of cell cycle events, the effect of cellular signaling, the dynamics of chromosome and spindle movement. Proteins involved in each of these events affect sister chromatid separation. During the cell cycle, sister chromatid separation occurs but once, after the S- and G_2 phase where cells have completed DNA replication but have not undergone mitosis. This is during the M phase. If DNA replication continues but sister chromatid separation does not occur, then cells containing many copies of each chromosome, polyploid cells, will be made. For organisms such as humans, this is not a nice thing to happen, but many plants and insects have polyploid nuclei as a normal part of their development. Alternatively, if sister chromatid separation occurs at a higher frequency than DNA replication, then cells with a reduced amount of DNA will result. This is certainly not a very nice thing to happen to a cell. Signals, responses, glues, motors, spindles: in studying the cellular orchestra that these players have organized, we are studying a very fundamental property of life – the ability to replicate and survive to replicate.

Amit Bardhan (**page 376**) addresses the mechanisms underlying the cohesion of sister chromatids during a process different from the relatively simple situations mentioned above. He focuses on meiosis, the events that take place to generate egg or sperm cells in which the chromosome number is half that of somatic cells. In this process too, sister chromatid cohesion and separation are important, critically so since the next generation is involved. Bardhan discusses evidence that suggests that different parts of the chromatids may have different mechanisms of adhesion and reviews the functions of genes that are involved in the process.

K. VijayRaghavan

Potential application of markers

Studies on the organization of genetic material, what it encodes and how it functions in the building of an organism: these are some of the areas that genome analysis addresses. Another major effect of technical advances in molecular genetic methods is the rapidity with which relatively crude maps of genomes can be generated. Given the fact that genomes of 'related' organisms are similarly organized in many respects, even a gross map can be very useful if it can be compared with a dense map in another organism. Thus, the study of how grasses have evolved and diversified has been greatly aided by the study of the genomes of wheat and rice. Similarly, the high level of conservation of genome organization between mouse and human, although they 'separated' about 100 million years ago, allow the functional mapping of human DNA sequences that regulate the activity of genes.

Jayasankar and Dharmalingam (**page 383**) report the first steps of the analysis of the genome of two species of fish. The mapping of characteristics along a linear piece of DNA, the chromosome, requires that the characteristics be identifiable as 'similar but different'. In genome analysis these characteristics are often fragment size and sequence variation at a particular genomic location. Thus, sequence similarity identifies a location and variation in the region can be used to map this location. The polymerase chain reaction (PCR) has been applied widely as a sensitive method for genome analysis studies. Its strength lies in high level of sensitivity of the method, thereby requiring a small amount of sample. This advantage lends itself to applications such as the study of populations in the wild where sample availability is likely to be limiting. The disadvantage of the method is also its sensitivity. Many factors in the reaction can cause small variations that are amplified by the sensitive method. Reproducibility, a key to precise mapping becomes difficult. There are other methods which can overcome this problem and these use sequences called microsatellites. The identification of sequences flanking microsatellites and the use of variability in these sequences between animals is a powerful tool for mapping experiments. Jayasankar and Dharmalingam's experiments are at a very early stage, but the wide use of such methods is bound to allow the rapid genetic analysis of fish. Groups worldwide, particularly in Tubingen in Germany, have made steady progress in the study of the zebrafish, *Danio rerio*. Studies on this model organism will help in the mapping of other fish, in manner similar to what has happened in mouse and human and in plants.

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Caste and disease

Duchenne muscular dystrophy (DMD) is a muscle degenerative disorder afflicting almost 1 in every 3500 males. This genetic disorder is linked to the Xchromosome and is transmitted from mother to son in a recessive manner. Individuals affected by DMD do not survive beyond their late teens. In the related disease, Becker muscular dystrophy (BMD) patients struggle along until middle age, with a life span of about 40–50 years. Genetic disorders are often amplified by selective breeding amongst restricted populations. DMD occurs at a significantly higher level in Indian migrants in UK as compared to other local and ethnic groups. The disease itself is primarily a consequence of deletions in the dystrophin gene, although the precise molecular mechanisms involving the protein dystrophin remain unclear.

The identification of the gene responsible for DMD and its localization on the X-chromosome, together with polymerase chain reaction methodology, now permit analysis of the population-based variations in the incidence and pattern of gene mutations. Mishra *et al.* (**page 395**) analyse the occurrence of DMD/BMD in different caste groups in Uttar Pradesh. They demonstrate a higher prevalence of the disease in *Brahmins* and *Vaishyas*, presumably a reflection of centuries of strictly caste-based marriages. Their results, however, raise questions regarding a prevailing hypothesis that 'deletional mutations changing the translational reading frame of dystrophin should cause the severe form of the disease (DMD), but deletions still maintaining the frame, result in the milder form (BMD)'.

The ready analysis of the gene responsible for DMD/BMD offers the possibility for genetic counselling. The fact that DMD/BMD is an X-linked recessive disorder, ensures that mothers act as carriers and sons have a high chance (50%) of being afflicted. In a social mileu that places a high premium on the male child, the problems of counsellors are obvious.

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CURRENT SCIENCE

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CORRESPONDENCE

Plutonium and thorium in the Indian nuclear programme

The paper by R. Chidambaram and C. Ganguly (*Curr. Sci.*, 1996, **10**, 21–35) discusses many aspects of plutonium and thorium utilization in the Indian Nuclear Programme. However, use of thorium is not viable due to some important physics aspects of thorium which were overlooked in the above paper.

1. It is well known that thorium is found in nature without any fissionable isotope. What is not emphasized is that its thermal absorption cross-section is three times that of ^{238}U. This implies that replacing uranium fuel by thorium fuel, in an uranium-fuelled reactor, would require three times enrichment of thorium (^{235}U, Pu or ^{233}U) compared to that of uranium, if nuclear characteristics of the reactor (e.g. burn-up in MWD/Te) are to be maintained. For example, a natural uranium heavy water reactor (PHWR) which uses 0.7% ^{235}U enrichment, would require 2.1% enrichment if thorium is used. Similarly, light water reactors (LWRs) which use about 3–4% enriched uranium would require 9–12% fissile material enrichment when thorium is used. Let us further quantify this statement. A 500 MWe PHWR, now being built at Tarapur site, uses 80 Te of incore inventory of natural uranium which contains 560 kg of ^{235}U. However, if it is fuelled by thorium, it would require about 1.7 Te of pure fissile material in the form of ^{235}U, Pu or ^{233}U. Not only that, a thorium fuelled reactor will also require equivalent out of core inventory of fissile material which would be always locked up in the reprocessing and fuel fabrication plants. Hence a thorium fuelled 500 MWe PHWR would require about 3.4 Te of pure fissile material to have the same fuelling characteristics as those of a natural uranium fuelled reactor.

   India has 78,000 Te of natural uranium according to the paper mentioned above. To burn even fifty percent of it, centuries would be required. If fissile material becomes available as freely as the paper mentions, then natural and depleted uranium would also be available without restriction. In that case there would be no incentive to utilize thorium. Hence, so long as uranium (natural or depleted) is available, thorium would never be used, even if fissile material becomes available without any restriction.

2. Apart from the above nuclear characteristics, two other nuclear properties require attention.

   a) Resonance absorption cross-section of thorium is much less than that of ^{238}U. It is because of resonance absorption of ^{238}U, natural uranium heavy water reactors use fuel channels separated from one another with large quantities of heavy water to slow down fission neutrons past resonances. As thorium would always require enrichment and does not suffer from resonance absorption like ^{238}U, pressure tube type heavy water reactors (with on-load fuelling) do not give any advantage to thorium utilization. If ever thorium is used, it would be used in light water reactors, where clustering of fuel rods and on-load fuelling is not done.

   b) ^{233}U is formed from absorption of neutron in thorium by an intermediate step which involves ^{233}Pa with half-life of 27.4 days. This necessitates removal of ^{233}Pa outside the reactor core for decay or to have a reactor of very low neutron flux (low specific power), as absorption of a neutron in ^{233}Pa is a double loss, loss of a ^{233}U atom as well as that of a neutron. If maximum possible production of ^{233}U is a requirement, then lowering flux in the reactor increases the capital cost of a project. Removal of ^{233}Pa actually means that fuel be removed continuously from the core for ^{233}Pa to decay. Such a possibility exists only with fluid fuelled reactors where the fuel is either in aqueous solution or in form of a molten salt. Several experiments on these concepts were done at the ORNL, USA without much success.

3. The paper mentions that thorium could be utilized in India through PHWRs using natural uranium, Pu–Th and finally ^{233}U–Th route. What does it mean? A 500 MWe PHWR uses 2200 Te of natural uranium in its 30-year life and produces 5.5 Te of fissile plutonium (70% load factor). Extending this fuel cycle to

10,000 MWe programme means utilization of 44,000 Te of uranium, which may be the maximum available for economic exploitation. One also ends up with only 110 Te of fissile plutonium.

This fissile plutonium could possibly be used to enrich thorium to produce ^{233}U. However, it should be noted that yield of ^{233}U is less than half the quantity of plutonium by weight, due to lower neutron production capacity of plutonium defined by its 'eta' in thermal energy range. It would take about 30 years for conversion of plutonium to ^{233}U by successive recycling. The end result would be maximum 55 Te of ^{233}U. As we have seen that a thorium–^{233}U PHWR of 500 MWe capacity would require 3.4 Te of ^{233}U, one would then be able to install about 8000 MWe under this fuel cycle scheme. Canadian studies have shown that it is not possible to design a self-sustaining thorium – ^{233}U based PHWR. Moreover, even for the sake of argument, if one assumes it is possible to design such a system, the end result of all these efforts would be 8000 MWe installed capacity, about half a century from now, provided 10,000 MWe PHWR programme based on natural uranium succeeds with very expensive fuel cycle costs due to low burn up of fuel and other properties of ^{232}U which is produced with ^{233}U and gives strong radioactivity. Are our efforts worth spending in that direction?

4. In case fast breeder programme already taken up in India succeeds, then there would be no incentive to use thorium as it brings down its very property, which is breeding. Fast reactors are Pu–U systems and would never be used for thorium utilization, till uranium is available.
5. In case unlimited availability of fissile material becomes possible due to success of accelerator breeding or dismantling of nuclear weapons, as the paper hopes, thorium may be utilized to use its good metallurgical property. In this case one would use once-through fuel cycle. Highly enriched thorium fuel would be irradiated to very large burn-ups of the order of 100,000 MWD/Te, and then disposed off without reprocessing. This could be done only in LWR reactors.

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Balakrishnan and Kakodkar reply:

1. It is precisely because thorium appears in nature without any fissile isotope that the first generation of nuclear reactors are all uranium-based, and not thorium-based. The thermal absorption cross-section of thorium is indeed higher than that of ^{238}U, but it does not mean that the initial fissile content that is needed with thorium is proportionately higher. Two facets of thorium fuel cycle that Rastogi has not touched are: a) the much higher reactivity worth of ^{233}U in thermal reactors, compared to that of ^{235}U, owing to the higher number of fission neutrons released per neutron absorbed by ^{233}U; and b) the substantially slower fall in reactivity as fuel burns in a reactor which contains thorium in place of ^{238}U. The initial fissile content in a reactor is not just to make the reactor critical but also to sustain a certain high level of discharge burnup of the fuel. Due to the much slower fall in reactivity with fuel burnup in a thorium-fuelled reactor, the initial enrichment needed in a thorium-fuelled reactor is not three times that needed with ^{238}U but lower. A much better basis of comparison would be life time fissile material requirements. Thorium-fuelled reactors permit much larger use of *in situ* bred fissile material. It has been shown by calculations that at high burnups, life time fissile natural requirements are in fact lower with thorium than with ^{238}U.

   What we should not lose sight of is the fact that we are endowed with a large thorium resource. In energy terms this resource is several times larger than any other indigenously available energy resource that is suitable for bulk electricity production. Several studies showing superior performance of thorium in a number of reactor systems are available and there is no doubt on this score. What is really needed is to develop the specific technologies suitable for thorium exploitation. This is where India will have to take initiative as no other country capable in nuclear technology has any urgency to do so because they have access to large uranium resources.

   It should also be clear that breeders (based on fission or non-fission route) are necessary for growth in nuclear power capacity in our country regardless of whether we are talking of use of uranium or thorium. (In fact we are talking of making use of both.) There is no clash between breeder development and developments in the area of thorium utilization. The sooner we do both, the better off we would be, in view of our large electricity needs.
2. a) While heterogeneity by way of making the fuel in the form of rods as used in both PHWRs and LWRs does give advantage in light of the resonance absorption phenomenon, having the bulk of the heavy water separated as moderator from the coolant heavy water in a PHWR when there is no such separation in a Light Water Reactor is due to the difference in the neutron moderation characteristics of light water and heavy water. It has nothing to do with resonance absorption or with the differences in the nuclear properties of ^{232}Th and those of ^{238}U. Thorium is known to work well in a number of reactor systems and calculations have shown several advantages with heavy water lattice arising out of better neutron economy.

   b) It is well recognized that removing the intermediate product ^{233}Pa from the active reactor core in order to increase its chance of decaying to ^{233}U rather than getting converted to ^{234}U is desirable and that realization is what led to the development of Molten Salt Breeder Reactor in the US and to the fluid fuelled heavy water reactor under the KEMA project in the Netherlands. In the present situation where these technologies did not mature due to other technological hurdles, the PHWR, the heavy water reactor system is much better suited to thorium fuel than the Light Water Reactor with its poor neutron economy and without any capability for on-load fuel reshuffling.

3. The concept of a PHWR started with natural uranium, gradually leading to a self-sustaining ^{233}U + thorium cycle by passing through a plutonium–thorium intermediate stage is nothing new. Our studies have shown that such a self-sustaining fuel cycle started with no external supply of fissile material will increase the PHWR-based nuclear capacity by about 50% compared to what can be achieved with the same quantity of natural uranium used in the once-through mode. What is more important to recognize is that the energy that one can extract by going into Th–^{233}U mode in PHWR is many times larger than without it. One should not confuse the question of growth with energy potential. For growth we need to breed fissile material fast. For realizing the energy potential of nuclear resources, we need to develop thorium utilization capability. We need to do both. When talking about thorium we are concerned with the latter.
4. It is clear that early generation fast reactors should use ^{239}Pu–^{238}U fuel system to enable faster growth of fissile material. That does not preclude beginning with thorium utilization which is equally well done in thermal reactors with perhaps much lower cost.
5. With the hypothetical situation of unlimited availability of fissile material, the question of whether one should opt for reprocessing the discharged fuel or go for a high burnup once-through cycle depends on many considerations. However, as far as comparison between PHWR and LWR is concerned, what can be said with absolutely no uncertainty is that a thorium fuel cycle is far more beneficial in a PHWR than in a LWR for the simple reason that all the neutrons that are absorbed by hydrogen and by the various reactivity regulating materials in a Light Water Reactor with its shut down refuelling will be profitably absorbed by ^{232}Th to produce ^{233}U in a PHWR where the deuterium that is present, instead of its lighter isotope hydrogen, does not absorb neutrons. Besides, in a PHWR with its on-load fuelling there is hardly any excess reactivity that is nullified by neutron absorbing poisons.

Further, from a long term perspective, the recycle option which we are adopting is environmentally far more benign as compared to the option of permanent disposal of fuel.

Thus, in summary, we have to reiterate that thorium has a very important role to play in the Indian nuclear power programme, and as far as its use in thermal reactors is concerned, there is no doubt that thorium is better used in heavy water reactors than in the light water reactors. Even if the high pressure coolant heavy water is replaced by light water due to other considerations, heavy water is certainly the better moderator that can give higher mileage out of thorium – not light water.

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ISRO spy scandal – An unending witchhunt

As individuals associated with the country's space programme over the last twenty years and more, we are deeply concerned about what has happened and is continuing to happen to one of our fellow scientists S. Nambinarayanan. We are choosing this route to express our concern because of our inability to intervene meaningfully in the complicated chessboard of moves and countermoves that seem to make inevitable the continued persecution and traumatization of a valued colleague.

Many of us have known 'Nambi' for over twenty years. He was the leader of the team of engineers sent to France for acquisition of liquid rocket technology. He led with great ability, ISRO teams vested with the responsibility of delivering the second and fourth stages of the PSLV project. He was also for a while the leader of the cryogenic engine project. Like all of us, 'Nambi' may have his share of virtues and faults, but we have always appreciated his deep commitment to technology development in the interests of ISRO and the nation. The arrest and interrogation of Nambi in the so-called ISRO espionage case and the attendant newspaper publicity have done immense damage to his morale and even more damage to his innocent family.

The CBI investigation completely absolving S. Nambinarayanan of any wrong doing and the verdict of the Chief Judicial Magistrate, Ernakulam discharging all the accused in the ISRO 'espionage case' are now on record. However the clearance recently accorded by the Kerala High Court to further investigate the case will drag Nambinarayanan and his family through another period of misery. From the record and the evidence behind the 'espionage case', there appears to be no basis for the continued harassment of a person whose innocence has been proven. The reasons for our statement are summarized below.

The major allegation against Nambinarayanan was that drawings and documents relating to the Viking engine and cryogenic technology were handed over to foreign/enemy countries in exchange for large amounts of money in US dollars, and that these took place at three different locations (Madras, Bangalore, Trivandrum) in January, June and September 1994 on specified dates and times.

The detailed investigation by the CBI established:

- that the alleged meetings between the various accused never took place, and the various accused were present elsewhere on the specified dates and times;
- that no documents were handed over;
- that no documents were seized from anyone;
- that an independent ISRO investigation also revealed that in the case of both the cryogenic and the Viking engines, no original drawings or documents of any significance were lost or were missing;
- that no documents of any kind or computer compatible tapes of any kind were shipped out of Trivandrum;
- that no money transactions took place.

The CBI investigations also reveal:

- that Nambinarayanan never met Ramon Srivastava, S. K. Sharma or the two Maldivian women;
- that accusations against Nambinarayanan regarding financial gains from espionage are baseless and as a matter of fact, his life style is very close to that of a poor person;
- that a lie detector (polygraph) test administered to Nambinarayanan by the CBI validates that he was speaking the truth.

There is therefore no evidence to prove that espionage ever took place or that documents or money exchanged hands or that the accused including S. Nambinarayanan benefited financially from these dealings. *In view of the evidence on record, oral as well as documentary, it seems clear that the allegations of espionage have been found to be false, and that the espionage story, both in its genesis and subsequent development had no basis in reality.*

As people who have been associated with technology development in India, we have not been able to understand the logic of what is supposed to have happened in the 'espionage case'. Clearly the acquisition of technology by any foreign power is quite a complex process. Experience indicates that even when drawings are acquired under open technology transfer agreements, their transformation into working hardware takes time, expertise and large financial outlays. The Maldivian women involved, their mode of entry into India or their activities, seem to be inconsistent with the aim of acquiring sensitive technology by any interested foreign power. ISRO does not classify working level documents as secret, top secret or confidential. An internal investigation carried out by ISRO has shown that there are no original drawings related to the Viking or cryogenic engines missing. In fact, in this case, even fabrication drawings, which ISRO routinely passes on to industry, are not missing.

In order to make out an offense under Section 3 of the Official Secrets Act of 1923, there must be sufficient evidence on record to show that any person acts in a manner prejudicial to the safety or interest of the state. Subsection 1 of Section 3 goes on to elaborate what these 'acts' are. We presume that the alleged offense involves:

- approaching, inspecting, passing by or being in the vicinity of a prohibited place;
- making sketches, plans, models or notes which might help the enemy directly or indirectly;
- obtaining, collecting, recording, publishing or communicating to any other person any secret code, password, sketch, plan, model, article, note or other documents or information that is of direct or indirect use to the enemy or which relates to the matter the disclosure of which is likely to affect the sovereignty and integrity of India, the security of the state or friendly relations with foreign countries.

The conclusions of the CBI investigation mentioned above have shown no evidence of any such acts. Indeed searches made by both the Kerala Police and the CBI have revealed no incriminating material or receipt of money from or by Nambinarayanan.

It is clear, that in this case, in view of the facts and circumstances and the legal position, no offence has been committed under the Official Secrets Act by Nambinarayanan.

Like many large organizations, the space organization tends to suffer from petty jealousies and professional rivalries that get translated into personal animosities. These 'internal' problems have so far not spilled over into national scandals involving personal lives and reputations. The 'espionage case' reveals that the country's space programme, or for that matter other strategic programmes, may no longer be immune to outside interference. These dangerous trends, if allowed to continue, can demotivate and demoralize the many hardworking and dedicated professionals who have made Indian achievements in these areas possible, usually for paltry compensations. Such actions are likely to derail these programmes and adversely affect the national interest more severely than any foreign hand.

The espionage case has now been going on for more than two years. The personal life of an important contributor to the space effort like Nambinarayanan has been given a rude jolt if not ruined. His family has been victimized and socially ostracized. Clearly he has suffered greatly and it is time that this 'charade' is brought to an end so that a hapless victim of the so-called espionage scandal can get on with what is left of his life and career. We trust and hope that good sense will prevail and that further harassment of a valued scientist will cease.

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(Former Chief Election Commissioner and Former Additional Secretary, Department of Space)

U. R. RAO
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Indian scientists win top UN awards

In a forthright editorial 'An uncertain future' (*Curr. Sci.*, 1997, **72**, 5), P. Balaram has painted a realistic scenario of Indian S & T. Even S. K. Joshi in his presidential address of the 1997 session of the Indian Science Congress in Delhi minced no words, when he said in the presence of the Prime Minister that reduction in R&D funding (in India) is a short-sighted policy and would weaken us devastatingly in the long run.

Against this backdrop, a unique hat-trick of global glory comes as a pleasant surprise. More importantly, these international recognitions are considered as good as Nobel prizes.

Specifically, UNEP's Sasakawa Environment Prize for 1996 of US $ 200,000 has been awarded to T. N. Khoshoo of the New Delhi-based Tata Energy Research Institute, for his outstanding contributions to the protection and management of the environment. For over three decades, Khoshoo has been an advocate of strong regional environmental planning for long-term ecological and economic security, particularly in the developing country context (*Our Planet*, 1997, **8**, 33).

The prestigious World Food Prize for 1996, also of US $ 200,000, has been awarded to G. S. Khush of the International Rice Research Institute, Manila, Philippines. World's premier rice breeder, Khush has spearheaded the Institute's programme for developing high yielding, disease and insect resistant varieties of rice. More than 300 improved varieties of rice have been selected and released by national rice improvement programmes from the material developed under the leadership of Khush over the last 29 years. These varieties and their progenies are now planted in about 50% of the world's rice area.

Eminent theoretical astrophysicist J. V. Narlikar, Director of the Inter-University Centre for Astronomy and Astrophysics, Pune, and Jiri Grygar of the Academy of Science, Prague, Czech Republic share the UNESCO's Kalinga Prize for popularization of science. Each year since 1951, UNESCO has conferred the £ 1,000 Kalinga Prize on an individual who had a distinguished career as a writer, editor, lecturer, radio/television programme director or film producer, and has interpreted science, research and technology to the public (*Curr. Sci.*, 1994, **66**, 618–620).

All said, these success stories of Indian scientists strongly suggest that given the right support, both financial and otherwise, we can do wonders. As India's first Prime Minister Jawaharlal Nehru has rightly said, 'It is science alone that can solve the problem of hunger and poverty... The future belongs to science and to those who make friends with science....' It is high time that the government doubled our R & D expenditure to mark the Golden Jubilee of Independence (1947–1997).

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On the recent editorials

P. Balaram in an editorial (*Curr. Sci*, 1997, **72**, 5) has rightly drawn attention to the declining funding and support for science and higher education in the era of liberalization. Though most of the arguments are balanced and reasonable, the reference to 'herbal petrol fiasco' is made rather naively. It was the immature and unscientific approach adopted by premier science establishments like DST and CSIR which primarily led to the public madness (see, for example, V. Ramamurthy's interview reported in *Nature*, 1996, **383**, 112). Readers may find it amusing to read a guest comment by M. Shermer (*Am. J. Phys.*, 1996, **64**, 1229–1230) the title of which conveys the essential point 'Hope springs eternal – Why people believe weird things' in the context of public perception of science.

I think the title of Balaram's editorial 'An uncertain future' and implied implications could be viewed differently: Is not an uncertain future challenging, worth enjoying, and full of new possibilities? Perhaps *Current Science* can make a virtue of this uncertain future by vigorously promoting informed debate and criticism of science establishment, and disseminating policy matters to the readership. It is worth cautioning against the pitfalls involved in the proposed thrust outlined in the editorial entitled 'Reaching out to industry' (*Curr. Sci.*, 1997, **72**, 97). One must be clear and careful regarding one's role: Why should a scientist be supposed to assume the mantle of an entrepreneur or a graduate physics syllabus be flooded with computer and electronics courses or an engineering graduate seek MBA? Journals like *Journal of Scientific and Industrial Research* ought to focus on 'developing a new synergism between academic science and technological innovation', and journals established for such specific purposes ought to be strengthened. Regarding industry–academia interaction, more than what is already within the scope of *Current Science* would be inappropriate and a short-sighted approach. Depending on the need and urgency, it would be advisable to launch supplement to *Current Science* specifically for industry.

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Hello Dolly!

K. P. Gopinathan

The recent creation of a true clone of a mammalian species in the laboratory, a sheep named Dolly, has evoked widespread interest and concern in all sections of people. The major question is how close are we now to generating human clones (see cover).

Hello Dolly, Well Hello Dolly,
It's so nice to have you back where you belong....
You are looking swell, Dolly, I can tell Dolly....

So goes the popular song from one of the greatest hits of Broadway, *Hello Dolly* of the 1960s. We say Hello now to another Dolly but this time near Edinburgh, Scotland. She has become the Darling of so many people already. Created by Ian Wilmut and his team of scientists at the Roslin Institute for Agricultural Sciences, Dolly is probably the first true clone of a mammal. The scientific team has developed a live sheep, now about 6 months of age, from a single cell derived from the udder tissue of a mother sheep by transplanting its nucleus into an egg cell from which its own original nucleus was eliminated. The egg cell containing the nucleus from the fully differentiated udder cell was then implanted into the reproductive tract of a foster mother, which developed to the normal baby sheep. The research paper published in *Nature* by Wilmut and coworkers[1] has raised many an eyebrow.

This indeed is a marvellous technical feat and an exciting piece of science but the hornet's nest has been opened. For, the major concern is – how far away are we from cloning of the human being? A Harvard University Professor is supposed to have written to *Nature* to stop the publication of this article, being genuinely concerned about its social and ethical repurcussions on our race. The US President Bill Clinton has already asked the 'Bioethics Advisory Commission' to review the implications on human beings and has banned federally-funded human cloning research, if any, till clearance is received. The French Government spokesman too has reacted in a similar way. The Pope and the Vatican also have expressed concern on the ethics of man interfering with God's work. Newspapers all over the world have carried stories on this 'development'. So, what is this excitement all about and do we really have to fear? Let us look at the scientific part first.

It is generally accepted that in multicellular organisms, every single cell contains the same set of chromosomes and therefore harbours the same information content. That it is so is established already in plants because from a single cell derived from any part of the fully grown plant a whole plant or tree can be regenerated. This capacity of a single cell to give rise to a whole organism, generally referred to as 'totipotency' was by and large considered to be restricted to the plants. How could a

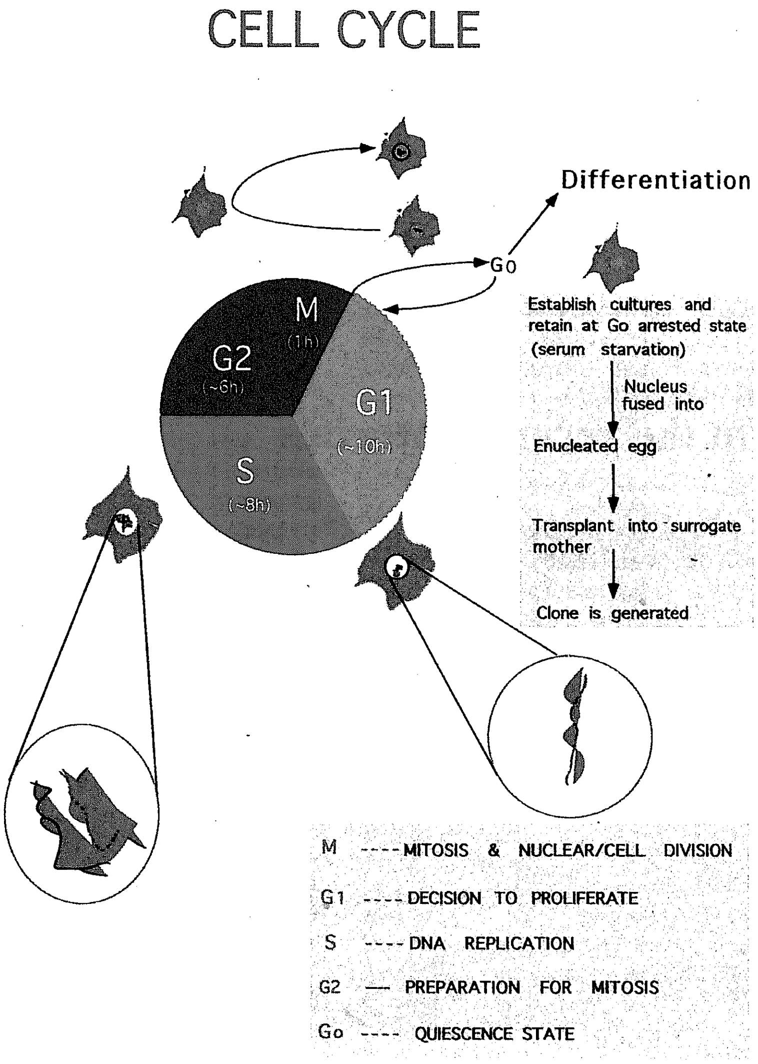
CELL CYCLE
Differentiation
Go
M (1h)
G2 (~6h)
G1 (~10h)
S (~8h)
Establish cultures and retain at Go arrested state (serum starvation)
Nucleus fused into
Enucleated egg
Transplant into surrogate mother
Clone is generated
M ----MITOSIS & NUCLEAR/CELL DIVISION
G1 ----DECISION TO PROLIFERATE
S ----DNA REPLICATION
G2 — PREPARATION FOR MITOSIS
Go ---- QUIESCENCE STATE

Figure 1. The cell cycle progression in a normal eukaryotic cell[8]. The shaded box on the top right outlines the approaches followed by Wilmut *et al.*[1], to generate the sheep clone, Dolly.

single cell from a fully differentiated organism derived from, say, the arms or legs of a human possibly give rise to a whole human being? It was widely assumed that such a feat will be impossible although theoretically conceivable. If one completely rips apart the whole human body, the entire set of cells numbering about 10,000 billion (10^{13} cells) should have the same set of chromosomes and genes each one harbouring the complete information to give rise to a whole human being. But how do we know that each cell contained the full complement of the genetic information? No one has sequenced so far the entire set of chromosomes/genes from different cells from a single individual or for that matter even from a single cell of an individual. The evidence is mostly circumstantial and deduced through indirect experimentations. As we know, the whole human body has been formed, after all, from a single egg cell derived from the mother following fertilization by a sperm cell originating from the father. The fertilized egg, the zygote, has gone on dividing to give rise to 2, 4, 8, 16, 32, ... and so on cells, each being the descendent of the immediate parent and consequently inheriting an identical daughter nucleus. In the development process, however, the axes in which the cell divides is an important consideration in the context of differentiation. Thus, the division of the embryonic cell on the X, Y or Z axes, decides the 'head or tail' (anterior/posterior), 'left or right' (proximal/distal) or 'front or back' (dorsal/ventral) development programming of the organism. It is clear therefore, that at precise points of time during the embryonic development, decisions are made about the fate of the cell and all its descendants then obey the order. At a molecular level, does this decision making involve a complete set of rearrangements of genetic information on each of the chromosomes within that cellular nucleus? For certain, we know that in the antibody producing white blood cells, the genome undergoes permanent rearrangements at the immunoglobin locus to give rise to only one particular type of antibody. If such rearrangements have taken place at other loci in other fully differentiated cells, it is possible that one can never retrieve the full genetic information to regenerate the whole animal from a single committed cell.

However, in the amphibians, as early as 1960s the Oxford Zoologist Gurdon[2,3] had demonstrated that if the nucleus derived from the mature intestinal cells of the adult frog is implanted into the enucleated frog eggs, the embryos developed into tadpoles and matured into frogs. But then, a frog is a frog is a frog! The experiment was widely appreciated by the scientists and hardly led to any serious reactions from the general public. In essence, the present set of experiments conducted by Wilmut and coworkers are similar except that the system explored is mammalian. Obviously, we have moved several steps closer to the possibility of doing such experiments with humans and that indeed is the concern. In fact, the developmental potential of mammalian embryonic nuclei (but not those from adult or fully differentiated tissues) was reported previously in the 1980s by Illmensee and colleagues[4] by transplanting them into mouse eggs and obtaining live born nuclear transplant recipients; however this claim has been contested subsequently[5–7].

What are the special features of the Wilmut experiments reported presently? The research team had earlier established that viable lambs can be produced by transplanting nuclei from very early sheep embryos (blastocysts) to enucleated eggs. They now demonstrate that the same procedure can be used to obtain viable lamb from a cell line that was established from the udder (a fully differentiated tissue) of a six-year-old ewe. In the past, the attempts to clone mammals have had mixed outcomes. Nuclei taken from mouse embryos older than 8-cell stage from the totipotent embryonic stem cells or from inner cell mass failed to produce viable embryos, although adult sheep clones were produced using nuclei from early morula (8–16 cell stage embryos). The major problem in getting the transplanted oocytes to develop properly was the cell cycle incompatibility between the donor nucleus and recipient oocytes (see Figure 1, for cell cycle progression). In mammals, most of the embryonic nuclei used as donors were in either the S or G_2 (non-diploid) phases of the cycle, which is not compatible with the diploid metaphase II-arrested oocytes, the preferred recipient stage. Additional rounds of DNA replication and premature chromatin condensation in such cells result in abnormal development. In the present set of experiments, the nuclei from cells that have been arrested in G_0 phase of cell cycle by serum starvation were utilized for transplantation which perhaps provided the most favourable conditions for the proper development of the embryos.

By these experiments, Wilmut and coworkers[1] have clearly established that during the growth and differentiation of an embryo, irreversible modifications of the genome do not occur in somatic cells. Nuclear transplantation from somatic cells could be used to produce clones of sheep that have been selected for particular traits, although the number of successful transplants is still low. This approach may prove to be of great use to produce sheep of desired germ line, though still not easily practicable. Newspaper reports have already appeared stating that at the Oregon Primate Research Center in the US, Don Wolf and his colleagues have established the clones of simians, although by a different approach. Here, instead of nuclear transplantations into the egg cells, the reimplantation of portions of blastocysts derived from a single fertilized egg into surrogate mothers has been successfully attempted to generate the clones. However, the latest newspaper reports state that the technique employed here was also nuclear transplantation. Have we thus moved a step closer to the cloning of the human race or is it still a bug bear? Technically, the feat is not going to be very different but the issue is an ethical one, as to how far we want to go. If one were to clone a particular human being with the 'desired traits', the first task is to address the question of what the desired traits are. Is it fair skin or blue eyes or blond hair, or is it a higher level of intellect that makes a better human being? Are the presence of genes alone enough to confer the behavioural traits of the clone and what role does the environment play to dictate his/her gene functions? The desire to clone the idolized 'father' by an overaffectionate son or daughter, or for that matter, of any other kith or kin, if achievable, should also address the matters related to personal relationships and emotional attachments. In the above instances even if the clone is generated through the successful embryonic development, will the emotional linkage between the individuals be sustained? In other words, can the soul be custom made? Duplications do occur naturally as in the case of identical twins, but the idea of a shadowy duplicate of oneself is undoubtedly a deeply disturbing thought. Are the clones of Frankensteins and Adolf Hitlers already in the production pipeline and if not can the days be far?

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Biogenicity of stromatolites and early life on Earth

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The earliest evidence for the origins of life on Earth go back to more than 3.5 billion years (b.y.) and this has been gathered from what is believed to be remnants of life on primitive Earth – certain odd dome-shaped structures called 'stromatolites'. These are actually made up of wavy and wrinkled layers, hardly 50–200 μm thick, and built out of fine marine sedimentary particles of silica, carbonates, barite or gypsum by microorganisms (bacteria, algae) layer upon layer (Figure 1). Earlier this century, these layered structures were mistaken as remains of other organisms like formaminifera, sponges and related marine species. However, their biological connections became established only after finding microbial fossils in some. Further, in modern times, cyanobacteria and algal communities are seen building up stromatolites off the west coast of Australia[1,2], and also in certain hot springs. Scientists were, therefore, convinced about the biogenicity of these primeval structures. Stromatolites have been reported from many parts of the world and some of the well-known occurrences are from Gunflint Formation in Canada (2.0 b.y.), Bulawayan Group in Rhodesia (2.5–2.8 b.y.), Pongola Group in South Africa (3.0 b.y.), Warawoona Group in Australia (3.3 b.y.) and in India they have been described from the Sandur region, Karnataka, dated around 2.9 b.y.

During the last few years, scientists have pushed back the beginnings of early life by reporting discoveries of microbial fossil-bearing stromatolites from the 3.4–3.5 b.y. cherts of Warawoona Group and Pilbara Block in western Australia[3,4], the 3.5 b.y. cherts in South Africa and 3.77 b.y. quartzites from Greenland[5]. Several of globally distributed Banded Iron Formations, some of them as old as 3.5 b.y. are themselves believed to be strong evidence for life, inasmuch as the conversion of iron to iron oxide in these formations requires free O_2 (absent 3.0 b.y. ago) which must have been supplied by O_2-producing microorganisms (cyanobacteria). These, no doubt point to the prolific activities of bacteria in those distant geological times and also imply that terrestrial life must have begun much earlier to have evolved sufficiently to build these organo-sedimentary stromatolites[6].

Though geologists were convinced about the role of microorganisms in building up the stromatolites and hence considered them as good evidence for life on primitive earth, doubts have now arisen whether some of the stromatolites can as well be non-biologic in origin, quite unrelated to life. This view has been particularly strengthened by the absence of fossil microorganisms in many stromatolites of Archaean age (4.5–2.5 b.y.), a view further supported by the latest observations by John P. Grotzinger and Daniel H. Rothman of MIT who found inorganic precipitates of aragonite and calcite on the ocean floor strongly simulating stromatolite structures[7]. The reliability of stromatolite as evidence for life has, therefore, come under a cloud and geologists have now felt a need for reexamination of the notable occurrences around the world as to their mode of genesis.

In their work, which has somewhat jolted the accepted notions, Grotzinger and Rothman have made critical morphological characterization of 1.9 b.y. old sub-tidal reef stromatolites from Wopmay Orogen, northwestern Canada; they examined the out-crops on the field and undertook laboratory measurement of thicknesses of calcite layers on polished samples and felt, on basis of microscopic textures and fractal dimensions, that these layered structures would very well be non-biologic or abiotic. According to the mathematical models they evolved using computer simulations, they could account for the development of these layered growth through the following four abiotic growth mechanisms: '(1) fall-out of suspended sediments; (2) diffusive smoothing of settled sediments (that is, sediments move downhill at a rate proportional to slope) and surface tension effects in chemical precipitation; (3) surface-normal precipitation; and (4) uncorrelated random noise representative of surface heterogeneity and environmental fluctuations'. The two, however, do not doubt the biogenicity of some of the world occurrences of stromatolites, but feel that in the absence of microfossils or other unambiguous clues in them, their biological origins cannot be taken for granted.

Grotzinger and Rothman's proofs about the abiogenicity of some of the stromatolites do not, however, rule out

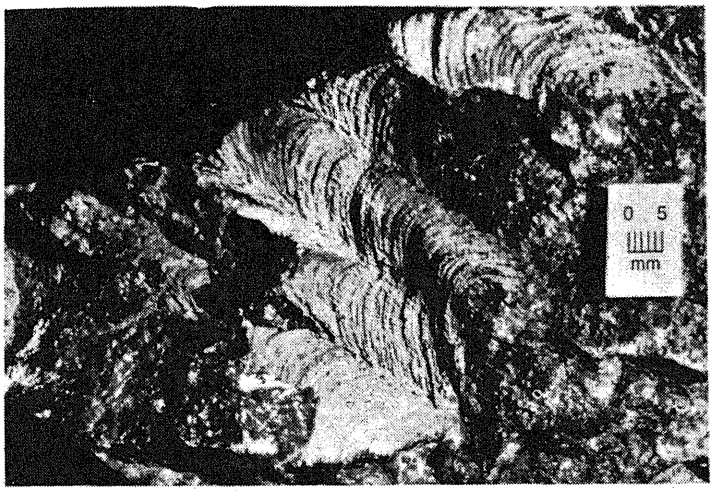

Figure 1. A typical wavy stromatolite from 2.5–2.8 b.y. old Bulawayan Group, Rhodesia, showing the finely-layered structures.

the prospect of determining existence of life during the pre-3 billion year period. Their work only underlines the need to have samples exhibiting undisputed features pointing to their organic links or preserving some typical biogenic signatures, which are neither degraded over time or altered by metamorphism and other geologic processes. A few researchers have been able to find such pointers and have undertaken studies using them to prove unambiguously biological processes at work during the early or primitive times. The studies of carbon residues, believed to have been derived from microfossils from 3.8 b.y. old quartzites from Greenland, is one example of an indirect evidence reported, after Raman microprobe studies on them revealed spectra typical of organic functional groups[5]. However, these findings have not apparently, received acceptance because of the possibility for alternate interpretations of the data. Likewise, scientists were skeptic about similar evidences supporting life processes from studies using sulphur isotope ratios (as evidence of microbial sulphate reduction) on 2.7–3.0 b.y. old samples[8].

In this context, a recent work by Stephen J. Mojzsis of Scripps Institute of Oceanography and his colleagues from California, Australia and UK[9] has come out with a more convincing evidence from 3.8 and 3.85 b.y. samples. They have taken recourse to the isotope ratios of $^{13}C/^{12}C$, as these values in the biologically-derived carbon are distinctly different from those from inorganic processes. Biogenic carbon, i.e. formed by natural decay of organisms, are enriched in the lighter isotope ^{12}C while the inorganically derived carbon have more of the heavier ^{13}C and hence the $^{13}C/^{12}C$ ratio can be used as a reliable indicator of parentage, whether biologic or nonbiologic.

Mojzsis and coworkers have done exactly this and took for isotope measurements 3.8 b.y. old Isua Formation (Banded Iron Formation or BIF) in west Greenland and a slightly older 3.85 b.y. specimen from nearby Akilia Island. They examined the carbon residues present in the form of graphite, unalterably locked up within apatite, a mineral found in these BIF. This mineral, $[Ca_5(PO_4)_3(OH, F)]$ a fluorophosphate of calcium, is a notable constituent of marine sediments where 'organisms participate in concentrating organic phosphate species from solution, and by recycling organic phosphate species from decaying P-rich bioorganic matter'; carbonate hydroxy fluorapatite is nucleated around calcium which these microorganisms can seggregate from water[9]. This microcrystalline apatite eventually gets diagenetically recrystallized to crystalline apatite while the inclusions of carbonaceous material are converted to graphite by later metamorphic event. In an earlier work on whole rock samples from the same area in Greenland, Manfred Schidlowski of Max Planck Institute for Chemistry found ^{13}C to ^{12}C ratio 1 to 1.5% lower than the international standard and ascribed this to origin from organisms[10]; but, others considered the difference too small and the values also, fell within the range usual for inorganically derived carbon. However, the carbon fractions Mojzsis and his colleagues examined were on inclusions from apatite, about 10 μm in size showing some 20 picogram of carbon, unaffected by later geologic activity thus preserving the pristine isotopic ratios. Using intense ion beams from an ion microprobe to penetrate the apatite, the authors extracted carbon ions from these graphite and thereafter determined the proportions of light and heavy carbon isotopes in the extract by mass spectrometry. They found that the ratios in these inclusions were 10 to 15 per cent lighter, well outside the range created by known inorganic processes, and hence 'characteristic of carbon isotopic compositions for organic matter'[9] and a strong evidence for life on earth as early as 3850 million years ago. Though electric discharges can alter carbon isotope ratios in organic material and incoming meteorite falls can contribute to light isotope enhancements, such possibilities are ruled out in Mojsis' samples in view of adequate evidences showing bolide impacts were much subdued by 3.8 b.y. (ref. 12).

Evidences in support of past biotic activity, therefore, must invariably have signatures unaffected by passage of time or later geologic processes. It is in this context that the determination of carbon isotope ratios on inclusions of carbonaceous materials, unequivocally biologic, well preserved within stable mineral structures, assumes importance. With the current availability of highly sophisticated analytical tools to extract and analyse extremely miniscule fractions of samples, sometimes as little as a few atoms, it is hoped that scientists may soon set at rest the ongoing controversies about biogenesis of stromatolites or about life on Mars[11].

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SCIENTIFIC CORRESPONDENCE

Possible involvement of surface ultra-structural features in utilizing the semi-conductive properties of pigments in chemically coloured insect cuticle

The insect integument is characterized by the presence of cuticle, an extracellular matrix. It is constructed of microfibrils made of chitin, which is a high molecular weight polymer composed of *N*-acetyl glycosamine residues, joined together by β-glycosidic linkages, organized within the protein matrix[1].

Insect cuticle may be transparent or coloured, depending upon its function in various locations of the body. The transparency of the cuticle covering the ocelli and compound eyes, for examples, is maintained to allow entry of light through it. The transmission of light through other photosensitive structures, such as the cuticular window over the pupal brain in some silk moths, is also through transparent cuticle. The importance of electron microscopical studies on the surface ultra-structural features of transparent cuticle in relation to their varied and extensive role as interference filter, anti-reflection device, reflector of specific wavelength, etc. has been amply highlighted in the literature[1,2–5].

As far as the coloured cuticle is concerned, it may have either chemical or physical colours. The physical colours of insect cuticle are the result of some optical phenomena such as diffraction, interference, etc. The chemically coloured cuticle on the other hand, is formed by internal infoldings of the cuticle, as in the case of coloured wing and integument of Lepidoptera and Hymenoptera respectively. Pigment granules in these examples (pteridines) are present within or above the epidermal cells. The melanin pigments on the other hand, are embedded inside the cuticle[6].

Although reports on involvement of surface ultra-structural features in various optical phenomena exhibited by transparent[2–5] and physically coloured cuticle[7] exist in literature, the same is not available for the chemically coloured cuticle.

Here, I report the unique surface feature of the yellow spots on the cuticle of a coccinellid, subcoccinella sp. with the help of scanning electron microscopy, which may be relevant to its functional significance. The elytral cuticle of the beetle was secured horizontally to brass-stub (10 mm dia; 10 mm height) and coated with gold in a JFC 1100 (Jeol) ion sputter coater. Observations were made with a Jeol JSM 35 CF scanning electron microscope operated at 15 kV.

The dorsal surface of the yellow spot is characterized by the presence of innumerable spherical bodies (dia = 0.8 µm) arranged in definite pattern (Figure 1). They are separated from one another by a space of about 0.2 µm in certain places, but in most of the parts, they are very close to each other and are intact. At places, the surface of the yellow spot contains some systematically-arranged short processes (spines), which are extensions of the cuticle. The spines are arranged in a circle around the rim of a large spherical body (10 µm in diameter), at a distance of about 15 µm from the small compact spherical bodies. Within the spherical body, there exist some sensilla, about 7 µm long and 0.8 µm broad. The sensilla protrudes from a socket,

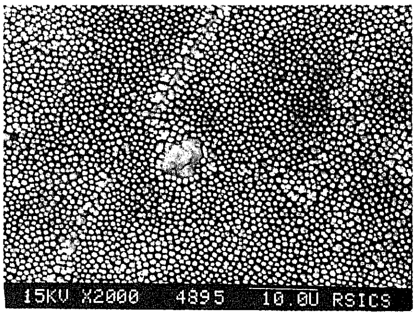

Figure 1. Scanning electron micrograph of the surface feature of the coloured spot (bar = 10 µm).

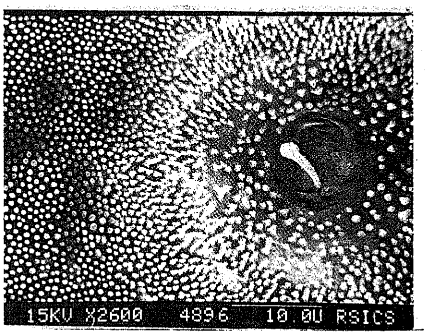

Figure 2. Arrangement of cuticular spines and sensilla (bar = 10 µm).

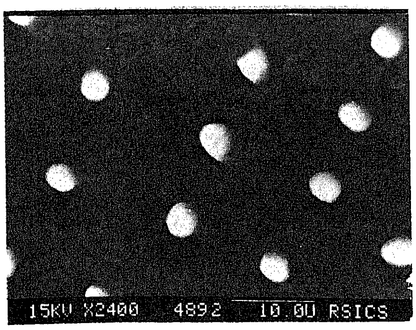

Figure 3. Scanning electron micrograph of the ventral surface of the coloured spot (bar = 10 μm).

about 1.6 μm thick, and tapers towards the tip with a thickness of 0.4 μm (Figure 2). It is interesting to note that the occurrence of intact small spherical bodies and the arrangement of spines and sensilla around a larger sphere is unique for the dorsal surface of the coloured spot. Further, the surface feature of the yellow spot, the location and arrangement of spines and sensilla do not show any individual variation in different specimens of the same species. A similar type of surface feature, however, is not observed on the inner surface of the coloured cuticular spot, which is smooth, but contains some cuticular processes (Figure 3). The general elytral cuticle other than the coloured spots is also smooth.

The spherical bodies on the external surface of the coloured cuticular spot appear to be related to the position and shape of the cells lodging the pigments responsible for the colour of the spot, since it is known that the surface pattern of the epicuticle reflects the outline of epidermal cells[1,8]. In fact, the structural pattern on the cuticular surface corresponds to the position of epidermal cells at the time of outer epicuticle deposition[9].

The intactness of the small spherical bodies on the surface of the coloured cuticular spot, suggests a similar kind of intact nature of the pigment cells in the epidermis. In this context, it can be mentioned that intactness of the tegumental pigment cells in some wasps was reported to be essential for photoconductive activity of the cuticle covering the pigment containing region[6].

Further, it is well known that many pigments such as melanins, carotenoids, haemoglobins and chlorophylls possess properties of organic semi-conductors[10], and are sensitive to changes in temperature and light intensity[11]. In the present case also, it is logical that the pigments associated with the cuticular spots serve as a sensitizer for light and temperature change. Such changes are expected to be transmitted to the nervous system through the sensilla associated with the pigment bodies. The changes in intensity of light and/or temperature are perceived by the sensitizing pigment, which transmits those changes to the cellular elements of the cuticular sensilla and thence to the nervous system.

A point of interest to be noted here is that, for conversion of heat and/or light into electricity, for sensing capacitive pressure, for detecting thermal conductivity and for sensing temperature, the present day electronic technology relies on semiconductor thin film device. The questions that are debatable in this context are, whether or not the semiconductive properties of coloured cuticle in general are utilized by the insects for sensory detection of temperature, light, humidity, pressure, etc. and whether or not they store the electric energy produced in the transduction process.

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SUDIP DEY

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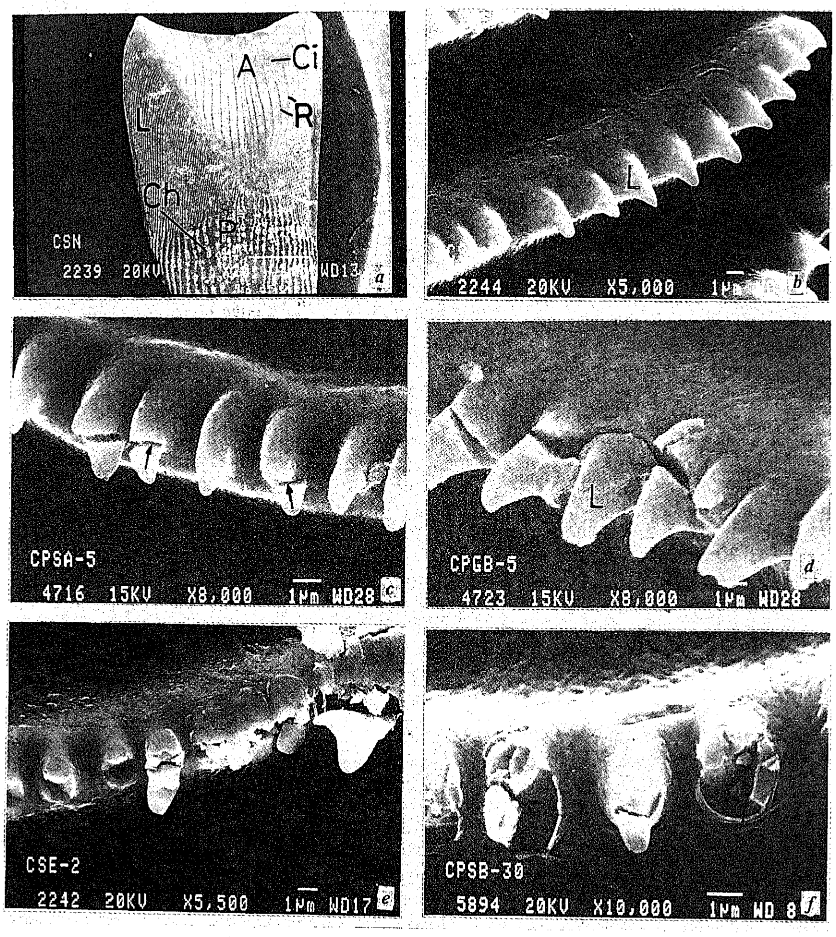
Lepidontal alterations of the circuli on the scales of freshwater snakehead, *Channa punctatus* (Bloch) upon exposure to malathion

Malathion, an organophosphorous insecticide, has been widely used to combat the attack of insect pests in northern India, without realizing the adverse effects on the aquatic organisms when it enters the waterbodies as run-off. It is a proven fact that it has higher toxicity even at low concentration levels to the aquatic organisms and comparatively lower toxicity to terrestrial mammals.

In the past, various organs have been employed (qualitatively and quantitatively) to assess the toxicity of various toxicants, but the use of scale as pollution indicator is of recent inception[1,2]. The detailed study of marginal circuli and mineral composition[1,2] of various parts of the scale of *Channa punctatus* (Bloch) has been used as an indicator of pollution upon exposure to different concentrations of endosulfan. We confirm here the earlier observations.

Live adults of *Channa punctatus* (29 g ± 1.58 S.D.; 4.4 cm ± 0.47 S.D.)


A
Ci
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2239 20KV WD13
a
L
2244 20KV X5,000 1µm
b
CPSA-5
4716 15KV X8,000 1µm WD28
c
L
CPGB-5
4723 15KV X8,000 1µm WD28
d
CSE-2
2242 20KV X5,500 1µm WD17
e
CPSB-30
5894 20KV X10,000 1µm WD 8
f

Figure 1 *a–f*. *a*, Scanning electron micrograph of normal scale of *Channa punctatus*. (Anterior, A; Posterior, P; Lateral, L; Chromatophore, Ch; Radii, R; Circuli, Ci); *b*, Normal lepidonts (L) attached on circulus of the scale; *c*, Lepidonts showing breakage (arrows) from the tip portion after an exposure of 0.05 mg/l malathion for 5 days; *d*, Lepidonts (L) in a row rooted out from the base (conc. 0.10 mg/l; 5 days). *e*, Complete damage of circulus showing lepidontal breakage (conc. 0.25 mg/l; 15 days). *f*, Disorganized lepidonts and circulus exposed at 0.10 mg/l malathion for 30 days.

were purchased from the local fish market of Chandigarh (76°46'30"E; 30°40'0"N). Fish were acclimatized for 7 days and fed once daily either with minced goat liver or live mosquito fish *Gambusia affinis patruelis* (Baird and Girard) collected from the pond located in the Botanical Garden of the Panjab University, Chandigarh. The LC_{50} value (96 h) of malathion (50% EC) for *Channa punctatus* was found to be 0.62 mg/l (ref. 3). The desired concentrations, i.e. 0.05, 0.10 and 0.25 mg/l of the pesticide in tanks were achieved by adding appropriate quantity of the stock solution. A parallel control group was maintained in toxicant free tap water (pH 6.8; dissolved oxygen 8.91 mg/l and total hardness 84 mg/l). A batch of 8 acclimatized fish was released in each tank of 20 l capacity. After 5, 15 and 30 days exposures, scales overlying the lateral line, leaving 7–8 dorsal fin rays, preferably from the second row were removed. These scales were sonicated for about 15 min to remove the extraneous material. Dried and cleaned scales were mounted on aluminium stubs, sputter coated with gold (100 Å thickness) and studied under vacuum using JEOL, JSM-6100 scanning electron microscope.

The cycloid scale of *Channa punctatus* (Bloch) is semioval in shape (Figure 1 *a*) and consists of three distinct parts, viz. anterior, posterior and lateral. The circuli are widely spaced on lateral sides and bifurcate when they enter the anterior region (Figure 1 *a*). In the posterior side, thick circuli are present. Each circulus in this region is formed by the fusion of 2–4 circuli present on the lateral side. This region also has several vertical rows of chromatophores. In the anterior region several radii, mostly tertiary ones, cut the circuli at a right angle. The SEM studies revealed that the anterior circuli bear rows of minute hook-like structures known as lepidonts (Figure 1 *b*). Lepidonts help the scale to anchor on fish body[1,4]. A lepidont is pointed at its free end and has a broad base. Each lepidont is firmly attached to the circuli (Figure 1 *b*). Initial observations revealed that upon prolonged exposure, one of the effects of toxicant is the loosening of the scale on the fish body as the main function of the lepidonts is to fix the scale on body of fish. The present study is restricted to discussing the toxicological impact of malathion on the lepidonts.

It has been observed that tips of approximately 5% of the lepidonts were broken from the anterior one third portion at 0.05 mg/l of malathion at an exposure of 5 days (Figure 1 *c*). With the increase in the concentration, i.e. 0.10 mg/l for 5 days, about similar percentage of lepidonts showed complete breakage from the base portion too (Figure 1 *d*).

Further increase in exposure period to 15 days at a concentration of 0.25 mg/l level of malathion caused complete damage to the circuli and the entire calcareous material became disorganized (Figure 1 *e*) accompanied by the loosening of the scale on the body, envisaging that the lepidonts play a significant role in anchoring the scale on the body of the fish.

Upon exposure for 5 days and 15 days to lower concentration levels of malathion, lepidonts beckoned less occurrence of breakage but with an increase in exposure duration to the extent of 30 days the frequency of the breakage showed drastic increase. At an exposure of 0.10 mg/l for a prolonged period of 30 days, 40% of lepidonts showed extreme breakage exhibited in various forms. Circuli on which the lepidonts are attached also showed certain level of damage (Figure 1 *f*).

Endosulfan[1,2] appears to be more toxic than malathion as even the lower concentrations of endosulfan damage both the circuli and lepidonts. The mineral composition of the different parts of the scale particularly the calcium also showed variations[2].

The present studies have clearly indicated that the lepidonts on the scale of *Channa punctatus* gave excellent response in the form of varying degree of damage to the varying concentrations of malathion. This damage is related to both, the dose and the exposure period. It is, however, difficult to conclude at this moment which one is more disastrous. Circuli showed less response at lower concentrations of pesticide exposure, but prolonged exposures certainly damage them too. Hence it can be concluded that lepidonts present on the circuli of the scale of *Channa punctatus* can be employed as a reliable pollution indicator with a high degree of exactitude.

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GENERAL ARTICLE

Conflict in science policy declarations: An analysis of the Indian model

Pawan Sikka

With a view to establishing a vision in India, policies, strategies and structures for science and technology (S&T) have been evolved, under a planned development approach since 1950. It is now observed that S&T policies, when considered in the overall economic milieu, have interacted with the trade, fiscal, industrial and political-policy instruments of the country. The need for evolving an integrated approach is stressed for making the science policy declarations of India as comprehensive as possible and goal-oriented towards attaining global scientific excellence. Developing countries can benefit from the rich experience of India.

APPRECIATING the role of science and technology towards economic development, policy making and planning exercise is undertaken and legislative measures are enacted for enhancing the country's potential. It may require consolidating and strengthening the input-resources like natural resources, manpower utilization, technological innovations and financial allocation. The need for science policy formulation and implementation is far more important for developing countries, than the developed ones, where the choice is between the planned development of science and no significant development at all.

Developing countries, which are now emerging on the world-scene of science and technology, could derive a benefit from the experience of the Indian model of socio-economic development based on science and technology, where policy measures were undertaken from time to time, during the past almost 50 years[1–6]. While charting the development of science in India, the following paragraphs would indicate areas of conflict in science policy declarations that would require some remedial measures and fine-tuning/adjustment, so as to meet with the global challenges *vis-à-vis* societal advantages.

Science in India

Policies, strategies and structures for S&T have been evolved in India, under a planned development approach since 1950 (ref. 6). The Government's attempts at science policy making in India are directed towards

- making S&T an integral part of all sectors of national activity for achieving self-reliance;
- developing capabilities essential for fulfilment of S&T tasks in these areas;
- undertaking of well-defined S&T missions; and
- provision of fiscal measures[7,8].

The Government of India, with a view to providing avenues for economic development on the scientific basis, adopted a Scientific Policy Resolution (SPR), in 1958, which provided guidance by directing the attention of scientists to some of the critical problems of society for which S&T could provide answers[9]. Besides, a number of policies have been adopted at the sectoral levels, in the recent years, to have an impact on the performance of S&T in the country, e.g. policies for energy, education, electronics and computer, environment, materials, drugs and chemicals, housing, patents and copyright, etc. (Table 1). Thus, the existence of the nature of Acts for the promotion and protection of S&T can be taken as an indication of the national commitment to the development of S&T towards the economic growth of the country[10].

Science policies declarations of the Government of India have played a dual role in order to

- foster and promote research activities in S&T in areas of interest and concern to our scientists, and at the same time to
- ensure that S&T are harnessed to programmes which are geared to fulfilling the needs of society.

Nehru employed the Scientific Policy Resolution of 1958, as Magna Carta for the development of science in India, while the visions of Shanti Swaroop Bhatnagar, Homi Bhabha and Vikram Sarabhai led to the establishment of a chain of national R&D laboratories in the fields of industrial, atomic and space sciences respectively[6]. The Government of India has assumed a major responsibility in supporting several initiatives and aspirations. This has resulted in

- providing enhanced budget for promoting S&T in the

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The views expressed here are the personal views of the author only and do not represent in any case the views of the organization to which he belongs.

Table 1. Emergence of science policy statements in India

Year	S&T policy declarations
1894	National Forest Policy
1927	The Indian Forest Act
1948	Industrial Policy Resolution (revised in 1956,1973,1977,1980,1990,1991)
1958	Scientific Policy Resolution
1968	Policy on Education
1970	The Patents Act
1972	Archival Policy Resolution
1978	Health for All (Population Policy)
1980	The Forest (Conservation) Act
1981	The Air (Preservation and Control of Pollution) Act
1982	National Health Policy
1983	Technology Policy Statement
1984	Ocean Policy Statement
	Computer Policy
	Telecommunication Report
1985	Textile Policy
	Policy Measures in Electronics
1986	Drug Policy
	National Policy on Education
	Computer Software Report
	Environment Protection Act
	R&D Cess Act
	Consumer Protection Act
1987	National Water Policy
	The air (Preservation and Control of Pollution – 1981) Act (revised)
1988	National Forest Policy of 1952 (revised)
	National Housing Policy
	The Water (Preservation and Control of Pollution – 1974) Act (revised)
1989	Motor and Vehicle Act
	Comprehensive Guidelines for Steel
1991	New Industrial Policy Statement
1992	Fertilizer Pricing Policy
	National Policy on Education, 1986 (modified)
1993	National Minerals Policy
1994	National Housing Policy
	New Drug Policy (revision of 1986)
	New Telecom Policy
	New Seed Policy
1995	The Technology Development Board Act (amended version of R&D Cess Act, 1986)
1997	New Textile Policy
	New Technology Policy (expected)
	Agriculture Policy (expected)
	Policy for Environment and sustainable development (expected)
	New Shipping Policy (expected)
	Patent Act modification (1970) (expected)

country, viz. Rs 20 crores in 1951–56 to about Rs.15,000 crores in 1992–96;

– creating infrastructure for the R&D establishments;

– establishing scientific, technical and educational institutes for generating the necessary S&T manpower, which now stands third largest in the world[11];

– supporting science in making all efforts so as to accrue all the benefits to the people by the application of S&T in India.

The pattern of development for further utilization of India's S&T resources, in particular, on measures for striking a balance between the domestic capabilities and foreign assistance has been the mandate of the apex science advisory bodies that has been identifying and recommending measures which would enhance the country's technological self-reliance with particular reference to Government's policy on foreign collaboration and import of technology[12]. With a view to achieving techno-economic development, India has adopted a two-pronged approach –

(i) development of indigenous R&D for building up an infrastructure for the transfer and application of technology towards industrial development; and
(ii) acquisition and transfer of technology from abroad and its gradual absorption, adaptation and dissemination in the prevalent system[13].

The development of S&T in India has been on the one hand, in relation to the great advances that have taken place in S&T in the world as a whole, and to the compulsion of ensuring that it is relevant and serves the overall purpose of the development of the country, on the other hand. The task has been formidable for a country like India with its large size and vast population. The efforts have met with real success in many areas and have provided experience in a few others – leading to capacity and capability buildings in S&T in the country.

Grey areas

The exchange of signals between the S&T policy regime and the other regimes has been influenced strongly by the perception of the political leadership and by S&T decisions on the nature and the extent of overlap between different regimes. These perceptions form constituencies of decision makers that are willing to effect adjustment and accommodation by accepting or ignoring signals from different policy regimes[14–16]. For example,

a) Table 1 indicates that there are some policy statements, while others are Resolutions and/or are Acts for the development and management of S&T in the country.

These declarations have different legal powers and jurisdiction and interpretations, whereas some of these have even non-statutory obligations[7].

b) Scientific Policy Resolution (SPR) was adopted in 1958, while the Technology Policy Statement (TPS) was announced, 25 years later in 1983, with the basic objective of developing the indigenous technology appropriate to national priorities and resources, thus leading to self-reliant economy in the country.

The SPR envisaged 'Effective utilization of the human and material resources through industrialization'.

The emphasis has been on the development of the scientific base, expertise, and scientific temper. Evidently, the object was to develop scientific capabilities and manpower as well as awareness among the general masses.

The TPS stressed on 'self reliance' based on protected economy which guided the planning as well as R&D involvement for the past decades or so.

In order to protect indigenous technology, it stipulated, 'Technology acquisition from outside shall not be at the expense of national interest. Indigenous initiative must receive due recognition and support'.

A draft paper for a new Technology Policy Statement was circulated, in 1993, to the scientific community seeking their comments, towards further strengthening the technology base and to assist the nation in fulfilling its role in the global economic environment with confidence and a sense of urgency. This is still under revision.

c) The Industrial Policy Resolution was first announced in 1948. It was later modified several times say in 1956, 1969, 1973, 1977, 1980, 1990 and 1991, keeping in view the changes taking place in the industrial scene abroad and fine-tuning it with the demands towards global competition.

Though New Industrial Policy (NIP), 1991 emphasizes self-reliance and development of domestic technology through investment in R&D, 'self-reliance' here implies the ability of Indian industry 'to pay for inputs through its own foreign exchange earnings'.

It expects 'foreign investment would bring attendant advantages of technology transfer, marketing experience, introduction of modern managerial techniques and new possibilities for promotion of export and inject the desired level of technological dynamism in Indian industry' and the 'Greater competitive pressure will also induce our industry to invest much more in R&D than they have been doing in the past'.

Clearly, in the absence of market competition, the Indian industries belied the expectation of the Government of India categorically stated in the TPS 1983, 'There shall be a firm commitment for absorption, adaptation and subsequent development of imported knowhow through adequate investment in R&D to which inputs of technology will be expected to contribute'.

The delicensing, deregulation, decontrol and abandonment of import substitution strategies adopted in the NIP envision progressively decontrolled market-driven economy and global technical interdependence. It is, thus, a complete departure from the earlier concept and in reality opens up the Indian industry to foreign investment and technological collaboration.

In a major shift from the policy on public sector, it specifies that 'public sector will continue to play a pivotal role in technology development and building of manufacturing capabilities in areas which are crucial in the long-term development of the economy and where private sector investment is inadequate'.

d) The Technology Policy Statement (1983) advocates for the selective import of technology for the promotion of indigenous one while the New Economic Policy (1991) stresses the need for the free/liberalized import of technology and technological products in the country.

e) The Technology Development Fund, launched in 1996 demands the payment of 5% of royalty on the import of technology (by amending the R&D cess Act of 1986) while there is no such levy on the import of new technological products – which are made liberal under the new economic policy of India.

f) India does not have any policy for environment protection while there exist a few policy measures as regulations/acts for the protection of air, water, forest resources, noise, etc. separately. Several laws relating to the management of environment and environmental resources do not clearly state the social objectives they aim to achieve. Consequently, the interpretation of the administrative machinery implementing the legislation is often not in conformity with the intent and purpose of the law.

Some laws, particularly on land use and management of environmental resources, appear to have self-defeating social objectives. Where such resources are shared by more than one state, legislation enacted in one state may have adverse environmental implications for a neighbouring one. India needs an integrated approach to its forestry, with a national, well thought-out policy if it is to undo the damage inflicted on its forest land heritage by a long legacy of neglect.

g) There are a few areas of contradictions or overlapping in the policy statements for the growth of industry and environment, environment and housing, forestry and paper industry, etc. For instance, the growth of human settlement and industrial expansion would cause scarcity on the available land-resources and affect the environment. Similarly, the expansion programme of textile industry needing bleaching and dying chemicals – the exodus of its waste water into river would cause pollution of the rivers – thus one impeding the growth of the other.

h) The attainment of self-reliance has been the motto before the policy-planners in India so far. Self-reliance has eroded the spirit of competition, while the present scenario demands targetting beyond self-reliance, resulting thereby in achieving technological excellence in the global market.

i) The absence of

- coordinating of scientific efforts and infrastructure,
- proper linkages among the academic, R&D and industrial sectors,
- goal-oriented policy towards commercial instinct of R&D efforts, and

- Prioritization in science and technology programmes, have crept in great measures in the S&T system, resulting thereby in its 'just' good performance[17].

Thus, various science policy declarations of India have indicated grey areas depicting a wide scope for new subject function oriented policy instruments. This points towards evolving a comprehensive S&T policy for the overall development of future S&T system in India.

Institutionalization of policy instruments

Science and technology policy and plans cannot succeed unless efforts are made to develop the necessary S&T infrastructure, for performing R&D, developing manpower, determining and deploying instruments, providing feedback, monitoring performance, performing technology assessment and forecasting, creating technology culture, coordinating R&D efforts and providing technological information.

The process of policy declarations and the institution-building, for implementation purposes, in science and technology in India has emerged, at different periods of time, respectively (Table 2). Normally, it is expected that when a policy statement is announced on a subject first, its importance and application should then find stress/focus in the current national Five-Year Plan, aspiring its implementation through the building of a scientific infrastructure in the country. But on the contrary, this approach has not happened in India and a 'disorder' in the S&T system has occurred, i.e. either a policy statement has appeared first, and/or the subject has not received enough promotion in the Plan Document and sometimes the institution has not been created to implement it immediately or vice-versa[17].

Besides, there are several subject-oriented laboratories which are not at all associated with the relevant/concerned Ministries. And, many like-mandated S&T institutions are preferring to work in isolation, rather than working in tandem – for unclear reasons. A national coordination body, say, a National Science and Technology Commission may be established[17,18] which could 'synchronize' the science advisory policy instrument and the creation of organizational structure with the national commitment in plan S&T component/emphasis as indicated in the national Five-Year Plans so that the entire science planning exercise gears towards accruing all the benefits of science and technology to the masses as well as leading to economic development in India. The present liberalized economic and industrial policy allows free import of technology and inflow of foreign investment. The Indian technologies are therefore facing greater challenges in the market. There is a need for an integrated approach for indigenous technology development, which would require study of laboratory level research and different aspects of scale up, technology transfer, commercialization and project financing. Such an exercise will boost the competitiveness of indigenous technologies.

Future perspectives

While examining the strength and weakness of the various science policy declarations of India, several questions have emerged on the surface, for example –

i) What is the relevance of these science policy declarations (Table 1) in the present context, which were announced from time to time, during the past 50 years? As a signatory to GATT/WTO, how will India introduce amendments in the Patent Law (1970) in product vs process patent regime, or Environment Protection Act (1986) in accepting the dumping of waste materials of the developed countries?
ii) How will the free flow of technology and investment from MNC affect the technology base in the country? implying India's emphasis on basic vs applied research in the future.
iii) How long would the R&D institutions act as satellite laboratories for the developed world in (a) carrying out the assigned jobs, (b) generating and supplying educated and trained technical manpower, and (c) preparing the qualified personnel to suit the employment requirements of MNC in India?
iv) When will India start employing/developing capabilities and capacities to manage the challenges of the frontier areas in science or new and emerging technologies?
v) Since economic and trade policies would now govern the bilateral cooperation in science & technology, industry, etc., further amendments would be needed in the policies which were framed yesterday to enable these to become strong and relevant today and tomorrow. (The recent American pressure on Russia not to affect the sale of MTCR technology to India, impeding India's rocket launching programme because of India procrastinating in signing NPT, GATT/(WTO)/Patent Law testifies the main statement.) There will be a demand for patent information after liberalization in India, with a view to developing and promoting indigenous technology and to avoid repetitive import of costly technology from abroad[19–22]. Besides, emphasis on R&D for technological innovations would aid India on the decreasing import of new generation technologies from abroad.

So, what would be the impact of liberalized economy for technology transfer and its commercialization in India over the existent technology base and spirit of the people? The S&T system would then require some fine-tuning in relation to the emerging scenario of international developments[23].

Table 2. Disorder in the institutionalization of Science & Technology policies in India

Policy declarations (year)	Creation of an institution (year)
Scientific Policy Resolution (1958)	Ministry of Science & Technology (set up in 1951, and 1985)
Technology Policy Statement (1983)	Technology Policy Implementation Committee (1984) Department of Scientific and Industrial Research (1985) Technology Information, Forecasting and Assessment Council (1988)
Environment Protection Act (1986)	Department of Environment (1980) (now) Ministry of Environment and Forest
Energy Policy Statement (1980)	Department of Non-Conventional Energy Sources (1982), (now Ministry of Non-Conventional Energy Sources)
Ocean Policy Statement (1984)	Department of Ocean Development (1981)
R&D Cess Act (1986)/ Fund for Technology Development and Application (1995)	Industrial Development Bank of India Department of Science & Technology (1971) Technology Development Board Act (1996)

To conclude, we need a reorientation of the policies to meet simultaneously the challenges of an internationally competitive economy and to provide satisfaction of rendering basic human needs to the population. An integrated science and technology policy must have three-fold objectives: Help India to emerge as an internationally competitive economy; effectively meet the needs of national security and be an active instrument of human emancipation; laying particular emphasis on meeting the basic needs of all our people[24]. Science and technology policies must be so designed as to actively assist India emerging as a strong and internationally competitive economy. The vision that must inspire us is that, in less than a decade, we should complete the transition from being a developing economy, to becoming a developed country, able and willing to face the challenge of global competition on an equal footing. Science policy in India has been evolved due to the appreciation of role of S&T for economic development by the political leaders as well as the generation of foresight studies which have pointed out towards the strength–weakness analysis of the existing S&T system and enabling India to undertake appropriate measures[25–27].

In order to attain global competence, an integrated approach to science policy declarations would be required, in respect of deciding priorities over capital intensive vs labour intensive technology – a factor that is very important for a developing country, like India, with meagre resources and large manpower. It would also require goal-oriented policy for setting 'targets beyond self-reliance', as the industrial scenario demands global competition and significant outward-looking economy, atleast in a few areas of techno-economic importance.

A coordinated and concerted effort among several policy measures when adopted, along with consolidated programmes and prioritization, for establishing a new science vision and yielding meaningful results for the prosperity of India, would be required for keeping alive her interests at the national and international levels – certainly an example for the developing countries to emulate for avoiding pitfalls!

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Sister chromatid cohesion during meiosis: Differential mechanisms for arms and centromere coherence

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Sister chromatid cohesion is a fundamental aspect of chromosome behaviour which ensures faithful disjunction of chromosomes. The differential release of centromeric- and arm-cohesion during meiosis suggests that the molecular basis of cohesion in the two domains might be different. A number of meiotic mutations differentially affect cohesion in the two domains. Recent identification of two proteins, ORD and mei-S332, shows that they are involved in maintaining cohesion in two spatially different locations. While mei-S332 acts to structurally hold the sister centromeres together until second meiotic anaphase, ORD is likely to time the release of cohesion along the arms during first meiotic division.

HAD there been no physical connection between the replicated chromatids, the so-called sister chromatids, recognition of the sisters and their subsequent segregation during divisional cycle would have been an extremely complex process. Perhaps to sidestep such a complexity, the cell has evolved an apparently simple mechanism by which the sister chromatids are kept in association right from their replication in S phase. The term sister chromatid cohesion refers to this physical bonding between the replicated sisters. Fidelity of chromosome segregation in mitosis relies on the maintenance of cohesion between the sister chromatids until anaphase transition. The tension generated as a result of the interaction of sister kinetochores with spindle poles and the opposing force imparted by cohesion (between the sisters) leads to the stable bipolar arrangement of chromosomes on metaphase spindle[1]; cohesion is relieved at the onset of anaphase to enable the two sisters to move to opposite poles. In contrast to mitosis where both the centromere and arm cohesion are dissolved simultaneously, the specialized cell division of meiosis requires a *differential release of cohesion* (Figure 1). Meiosis involves two rounds of nuclear divisions following a single premeiotic S phase and results in halving the chromosome number. During the first (reductional) meiotic division homologous chromosomes segregate from each other, while during the second (equational) division of meiosis, sister chromatids disjoin. Cohesion at the centromere is maintained throughout the first meiotic division so that the two sisters are constrained to move to the same pole during anaphase-I, and only at the onset of anaphase-II the centromeric cohesion is released allowing the sisters' migration towards opposite poles. On the other hand, cohesion along homolog arms helps stabilize and maintain chiasma position[2,3], thereby enabling the bivalents to achieve a stable bipolar orientation on the metaphase-I spindle to secure reductional disjunction during anaphase-I. The arm cohesion is dissolved at metaphase-I/anaphase-I transition presumably for the release of chiasmata. Thus, sister chromatid cohesion has a two-fold significance in meiosis as compared to mitosis, to secure reductional disjunction of homologs during first meiosis and also equational segregation of the sisters during the second meiotic division, and a defect in either of the two might lead to aberrant segregation resulting in nondisjunction.

A variety of mechanisms have been proposed to explain cohesion between sister chromatids. The hypothesis that the late replicating centromeric DNA might serve to hold the sisters together proved to be untenable with the finding in budding yeast, *Saccharomyces cerevisiae* that centromeric DNA is fully replicated well ahead of the time of chromosome segregation[4]. The idea that catenation produced during DNA replication could play a role in physically holding the chromatids together[5] has received experimental support with the finding that DNA topoisomerase II (the enzyme which reversibly untangle interlocked DNA duplexes) activity is required for anaphase transition[6,7]. Also, topoisomerase II has been shown to be required for segregation of recombined chromosomes during first division of meiosis[8]. In addition to catenation that interlock two DNA helices (comprising the two sister chromatids), some protein(s) may act to reinforce cohesion as suggested by recent evidences, and they may function either as structural proteins gluing the sisters together, or as regulatory proteins in timing the release of cohesion.

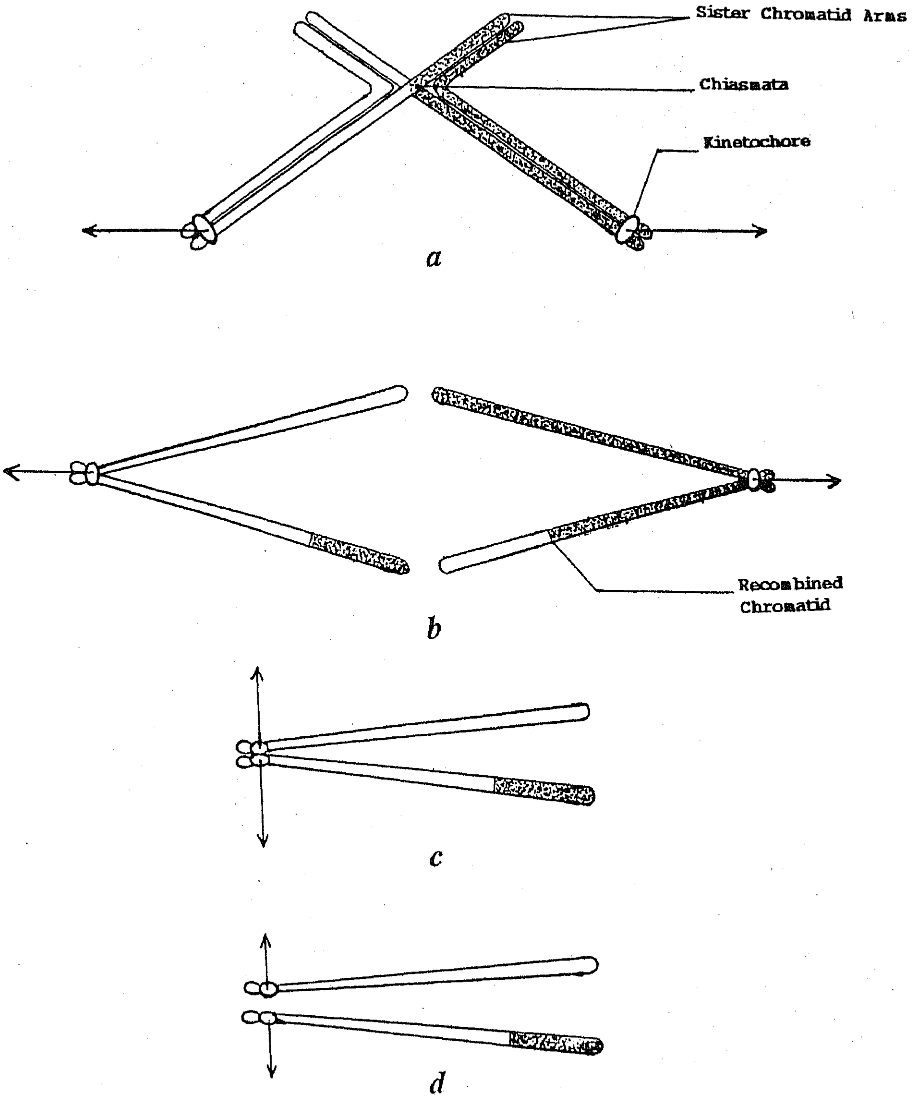

Figure 1. Chromosome behaviour during meiosis. *a*, A pair of homologous chromosomes (bivalent) connected by chiasmata, at metaphase-I. Sister chromatid cohesion is maintained both at the centromeres and along the arms; *b*, At anaphase-I, arm cohesion is dissolved (indicated by the gap between the chromatid arms) allowing the release of chiasmata, and the two homologs move to opposite poles; *c*, At metaphase-II, sister kinetochore differentiation occurs and they capture spindle microtubules from opposite poles; *d*, At anaphase-II, cohesion between the sister centromeres is released, allowing the sisters' migration towards opposite poles. In *c* and *d*, only one of the two homologous chromosomes is shown. In all the figures, arrows indicate the direction of 'pull' exerted on the kinetochore by spindle microtubules.

They include the gene products identified by a number of mutants defective in sister chromatid cohesion, and proteins identified by antibodies (see ref. 9 for a review). While catenation could provide the physical basis of cohesion that is common to both mitotic and meiotic chromosomes, the function of these proteins might be specific to either of the two divisions.

The differential release of centromeric and arm cohesion during meiosis suggests that different proteins might be involved in maintaining cohesion along the chromosome arms and at the centromere, and their mode of action might be mechanistically different. Alternatively, the same protein(s) might be shared in both the domains and only their removal/destruction is regulated differentially. That the molecular basis of cohesion at the centromere and along the arms could be different is also evident from the phenotypes of *Drosophila pim* and *thr* mutants. The *pim* and *thr* gene products are specially required for the release of cohesion between sister centromeres, but not between the arms, during mitosis[10,11].

This review addresses the probability that the mechanism of maintenance of cohesion might be different at the centromeres and along the chromatid arms, at least during meiosis. To do this, we will survey some of the potential candidate cohesion genes which act in meiosis and try to examine whether their action is restricted to either of the two chromosomal domains (i.e. the centromere and the chromatid arms). We then discuss the identified cohesion proteins and the possible mechanisms of their action. Though the gene products which affect cohesion during mitosis are significant in their own right, they will not be discussed here for the sake of brevity.

Potential sister-chromatid cohesion mutations

Non-disjunction in meiosis-I might occur in either of two ways: absence or reduction of reciprocal genetic exchange (for a review, see ref. 12), or failure in maintenance of chiasmata. Although the mechanism of chiasma maintenance is still a matter of dispute (reviewed by Carpenter[13]), cohesion between sister chromatids could be one of the major contributing factors[3]. The reduced genetic exchange, or early loss of chiasma (or both), could lead to formation of univalents in diakinesis/metaphase-I and subsequent random movement of the univalents on first meiotic spindle. On the other hand, mis-segregation in meiosis-II might result from precocious release of cohesion between the sister centromeres followed by their independent movement, and an earlier incidence of failure of genetic exchange does not seem to affect anaphase-II segregation. However, it is known that presence of univalents in metaphase-I spindle could delay anaphase transition and the univalents could segregate equationally; the resulting sisters again mis-segregate during second meiosis. Therefore, it seems difficult to specify whether a nondisjunction event results primarily from a failure of cohesion, or is a manifestation of reduced genetic exchange. The cases where recombined chromosomes mis-segregate might be informative, and could be the result of precocious dissociation of sisters, since here the resulting (from recombination) chiasmata fails to bind and (thus) to regularize disjunction. However, an early dissociation (between sisters) event could affect recombination by affecting homolog association, thus making it probable that mis-segregation of non-recombinant chromosomes could result primarily from lack of cohesion instead of lack of genetic recombination *per se*. Identification of genes which regulate meiotic cohesion has been done by isolating mutants that mis-segregate during meiotic division(s). Though a direct visual observation of chromosome behaviour in mutants could undoubtedly demonstrate the involvement of a specific gene in cohesion, identification of genes has been hindered by poor chromosome morphology in systems which offer a powerful genetic tool, e.g. the yeast and *Drosophila*, although in the latter case the male meiosis has made cytological approach amenable. Therefore, to understand the involvement of specific genes in cohesion, we will survey some meiotic mutations (Table 1) which result in mis-segregation during meiotic division(s), and examine the likelihood that the primary defect is at the level of sister chromatid cohesion. Special emphasis will be put on situations where recombined chromosomes mis-segregate, and, where recombination frequency is not significantly reduced by the mutation.

A number of mutations show a rather pleiotropic effect, affecting frequency of genetic recombination and synaptonemal complex (SC) structure/function, besides affecting chromosome disjunction during meiosis. This group includes the *red1*, *rec8*, *spo76* and *ord*[14–21]. All of them produce highly aneuploid meiotic products. Following the segregation behaviour of a number of centromere-linked heterozygous markers, it has been found that the mutations primarily affect reductional (meiosis-I) disjunction. Majority of the disomes were heterozygous for the centromere-linked markers, suggesting that the mutations affect meiosis-I segregation (non-disjunction in meiosis-II would result in disomes with sister centromeres, rather than with homologous centromeres). Though the mutations reduce frequency of meiotic recombination and thus raise the possibility that non-disjunction results from lack of genetic exchange, genetic analyses have shown that even recombined chromosomes undergo mis-segregation in the mutants[14–16,19,21]. The latter observations suggest that mis-segregation results from failure in maintaining chiasmata which, in turn, might be the consequence of precocious release of cohesion between the sister chromatids early in meiosis-I. Fluorescent *in situ* hybridization analysis of meiotic prophase chromosomes in the *rec8* mutant of fission yeast has shown that sister chromatids get frequently separated during first meiotic prophase[16]. Cytological observations in *spo76* and *ord* mutants also show precocious separation of sister chromatids in early prophase-I of meiosis[18,20,21]. It has also been shown that meiotic synapsis is aberrant in the mutants with defective SC formation[15,18], except in *ord* where synaptic progression has not been analysed. Even in *ord* it is highly probable that meiotic synapsis gets affected, since the mutant reduces recombination frequency. In fission yeast where classical tripartite SCs are not formed, and instead a structure called linear elements (which are thought to be equivalent structure to the lateral element of SC of other organisms) are formed along the length of meiotic prophase chromosomes, formation of the latter also gets affected in the *rec8* mutant[16]. One reasonable possibility could be that component(s) of SC confer sister chromatid cohesion (as suggested by Maguire[22–24]), and deformed (or absence of) SC formation reduces the probability of meiotic recombination. Alternatively, it may be possible that sister

Table 1. Features of the mutations

Mutation	Level of mis-segregation	Recombination frequency	Type of mis-segregation	Meiotic synapsis	Somatic mitosis	Others
Saccharomyces cerevisiae						
red1	Meiosis-I	Reduced	Both recombined and non-recombined chromosomes	No SC assembly	Unaffected	
med1	Predominantly meiosis-I, also meiosis-II	Reduced	Recombined and non-recombined	Normal	Unaffected	
dis1	Meiosis-I	Unaffected	Recombined	Not detected	Increased non-disjunction	
Schizosaccharomyces pombe						
rec8	Meiosis-I; also meiosis-II?	Reduced	Recombined and non-recombined	Aberrant linear element formation	Unaffected ?	
Sordaria macrospora						
spo76	Meiosis-I	Reduced in heterozygotes	Recombined; also non-recombined?	Aberrant	Unaffected ?	Defective in UV damage repair; no meiosis-II
Zea mays						
dy	Meiosis-I; also meiosis-II?	Unaffected	Recombined	Precocious desynapsis	Unaffected ?	
Drosophila melanogaster						
ord	Predominantly meiosis-I	Reduced in females	Recombined and non-recombined	Not detected	Unaffected	Gonial mitosis slightly affected
mei-S332	Predominantly meiosis-II	Unaffected	Recombined	Not detected	Unaffected	Gonial mitosis slightly affected

chromatid cohesiveness is needed for proper homolog association and SC formation, and a defect in the former could lead to aberrant SC formation with reduced genetic recombination. However, the pleiotropic nature of the mutations suggests that they might as well be some regulatory components of meiosis, parallely affecting different meiotic events independent of each other. Indeed, isolation of one allele of *ord* (*ord*^{6}) that affects recombination to the same extent as do the other alleles but affects disjunction less frequently[21] suggests that the gene can differentially influence the two processes, strengthening the direct involvement of the gene in maintaining cohesion.

The yeast *med1* mutation is also somewhat similar to the above discussed ones in the sense that it results in high level of meiosis-I, and to some extent meiosis-II, non-disjunction, and also reduces frequency of recombination[25]. But, in contrast to the mutations already described, SC formation is apparently normal in the *med*^{1} mutant[25]. Increased spore viability of *med1 spo13* double mutant suggests that *med1* function is required for reductional division of meiosis[25] (the *spo13* mutant undergoes a single meiotic division of predominantly equational nature; thus, meiotic lethal mutants which are defective in recombination produce viable meiotic products in a *spo13* background since *spo13* skips first meitoic division[26]). Although reduced recombination elevates meiosis-I non-disjunction in the mutant, a good number of the disomes result from precocious separation of recombined chromatids followed by their random segregation through both meiotic divisions[25]. Though it has been suggested that the defects in cohesion as well as recombination are secondary consequences of perturbed chromatin structure caused by the mutation[25], it cannot be ruled out that MED1 is a regulatory protein independently affecting cohesion and recombination. It may even be possible that MED1 function is required specifically for arm cohesion and a defective cohesion along the arms reduces probability of meiotic recombination by affecting homolog association in early meiosis-I. The *med1* mutant phenotype appears to be very similar to that of *ord* in *Drosophila*. Cloning and sequencing of *med1* will show whether the gene is homologous to the *Drosophila ord* gene.

Another good candidate for meiotic cohesion is the maize *dy* gene. In the homozygous *dy* mutants, sporadic precocious desynapsis of bivalents follows apparently normal pachytene synapsis. By diakinesis, bivalents often separate to univalents which lag for some time on the spindle and then segregate equationally during anaphase-I[27,28]. Frequency of meiotic crossing-over appears to be unaffected in the mutant (a distal heterozygous marker, a heterochromatic 'knob', was found to be separated equationally in most of the cases, confirming the occurrence of an exchange event between the knob and the centromere[28]). The inferred crossover frequencies in the mutant were equal and consistent with the expected wild type crossover frequency. The results show that the mutation has no apparent effect on meiotic recombination but it affects maintenance of chiasmata. Ultrastructural observations of synaptic progression showed that the central region of the SC disintegrates early, causing precocious desynapsis in the mutant. It has been suggested that one of the late functions of SC is to provide for sister chromatid cohesion, and that the lack of sister cohesiveness results in early loss of chiasmata[22–24, 27–29].

The yeast *dis1* and *Drosophila mei-S332* mutations apparently affect cohesion specifically at the centromere region, but the time of their expressions differs. While *dis1* predominantly affects meiosis-I segregation[30], *mei-S332* impairs meiosis-II disjunction[20,31,32]. Both the mutants have no apparent effect on recombination[30–32], suggesting that they do not interfere with homolog association, and non-disjunction involves almost all the chromosomes of the complement. Genetic analysis in *dis1* suggests that non-disjunction results from precocious separation of sister chromatids at meiosis-I followed by their equational, rather than random, segregation[30]. Cytological observation of meiotic segregation in *mei-S332* mutant males reveals that sister chromatids frequently dissociate in mid- to late-anaphase-I, resulting in non-disjunction or lagging chromosomes in anaphase-II[20,31,32]. The results suggest that although both the mutations affect specifically the centromeric cohesion (disrupted cohesion along the arms would reduce the probability of exchange event), the mechanism of their action most likely differs. *Dis1* might act to regulate functional differentiation of sister kinetochores, and an early differentiation in the mutant leads to premature separation of sister chromatids (if *dis1* were to maintain cohesion after sister kinetochore differentiation, the mutant would cause only meiosis-II non-disjunction). On the other hand, *mei-S332* is likely to act to physically hold the sister kinetochores, after their functional differentiation at prometaphase-I and early anaphase-I. *Dis1* differs from *mei-S332* in another respect, in that, it induces high level of mitotic non-disjunction as well[30], suggesting that *dis1* function might be shared between the two types of nuclear divisions.

It seems probable that a number of mutations could affect cohesion along the chromatid arms during meiosis, viz. *red1*, *spo76*, *med1*, *rec8*, *dy* and *ord*. All of them (except *dy*) reduce frequency of meiotic recombination, and also affect maintenance of chiasmata. This is expected from an aberrant association between the arms of sister chromatids during meiosis-I. A few of them might also act to maintain centromeric cohesion. However, it seems untimely to draw any conclusion about their involvement in centromeric cohesion, except to mention safely that if they are to maintain cohesion at the centromeres, the mechanism might be different compared to what operates for arm cohesion (discussed later).

Molecular mechanism of cohesion during meiosis

Recent identification of two proteins from *Drosophila*, mei-S332 and ORD, which are shown to be involved in maintaining sister chromatid cohesion in meiosis has provided a critical insight into the molecular basis of cohesion[33,34].

Kerrebrock *et al.*[33] have cloned the *Drosophila mei-S332* gene and have shown that the gene encodes a novel 44 kDa protein. Genetic and cytological analyses suggest a role for the protein in promoting cohesion specifically at the centromere region, after differentiation of sister kinetochores. This has been corroborated by the localization of the mei-S332 protein (fused to jelly fish Green Fluorescent Protein) on meiotic chromosomes. The localization pattern of the protein (on meiotic chromosomes) is consistent with the idea that the protein functions as a structural component to hold the sister centromeres together. Mei-S332.GFP localizes to the centromeric region of the meiotic chromosomes, starting from prophase-I onwards until metaphase-II (ref. 33). Strikingly, at the onset of anaphase-II the protein suddenly disappears from the centromeres and is no longer detectable[33], which is consistent with the requirement for the release of cohesion if the protein is to act to physically hold the two sisters together.

The *Drosophila ord* gene has been cloned and shown to encode a novel 55 kDa protein[34]. *Ord* transcribes only in adult ovary and testis, and in no other tissues or developmental stages, suggesting its meiosis-specific function. The low abundance of *ord* message suggests that the protein is likely to maintain cohesion in a regulatory manner, rather than as a structural component[34]. The effect of *ord* mutation on meiotic recombination is consistent with a regulatory role of the protein. Some of the *ord* alleles exhibit a unique phenomenon of negative complementation where one allele poisons the activity of another. When the mild allele ord^4, which retains a high amount of residual activity even in *trans* to a deficiency for the locus, is placed *trans* to some other *recessive* alleles (ord^1, ord^2, ord^6), the level of malsegregation increases significantly[34]. However, other recessive alleles (ord^3, ord^5) do not interfere with residual function of ord^4. Sequence analyses have shown that in ord^1, ord^2 and ord^6 (which impair ord^4 function), the mutations residue in the C-terminal halves of the proteins, while the ones that do not interfere with ord^4 function lack the C-terminal halves from missense mutations. This suggests that the C-terminal half of the protein is crucial for its function. Bickel *et al.*[34] have suggested that functioning of ORD requires protein–protein interaction through C-terminal halves. They have proposed that the ORD protein is having two functional domains, one for protein binding and another for exert-ing cohesion function, and that cohesion requires binding but binding does not ensure cohesion (see Figure 5 in ref. 34). Since ORD dimerization could not be detected, it is most likely that the protein interacts with some other protein molecule, which is yet to be identified[34]. The alleles which poison the activity of *ord*[4] might have altered 'cohesion domain' but intact 'protein binding domain' and form non-functional complex with the interacting partner, thus outcompeting *ord*[4] (which might have slightly altered 'protein binding domain' but intact 'cohesion domain') to form functional complex with the protein partner[34].

Another protein, Cor1, isolated from hamster has been suggested to maintain cohesion on the basis of observation that it localizes between sister chromatids of meiotic chromosomes (but not mitotic chromosomes)[35]. Cor1 is a component of the lateral element of SCs (ref. 35), and its localization pattern on meiotic chromosomes is consistent with a role of the protein in maintaining cohesion until second meiotic anaphase. Cor1 has been shown to localize axial to metaphase-I chromosomes with a substantial amount in association with pairs of sister centromeres. Interestingly, the centromeric Cor1 persists up to metaphase-II and dissociates at the onset of anaphase-II, which suggests that the protein might have a role in ensuring co-segregation of sister chromatids at anaphase-I (ref. 35). However, the evidence is rather correlative and it seems difficult to analyse directly the involvement of Cor1 in sister chromatid cohesion. However, the large body of circumstantial evidences for a role of some SC components in imparting cohesion is consistent with the role attributed to Cor1.

Concluding remark

A handful of potential candidate genes have been identified from diverse organisms, but till date only two candidate proteins could be identified which are unambiguously involved in maintaining sister chromatid cohesion during meiosis. With the identification of mei-S332 and its localization on meiotic chromosomes, it is evident that this particular protein is required specifically to maintain centromeric cohesion during meiosis, and the gene product has no apparent contribution in maintaining arm cohesion. On the other hand, the *ord* mutant phenotype manifests at a time when arm cohesion is very crucial (for maintaining chiasmata), and it is most likely that the ORD protein acts to maintain cohesion at least along the chromatid arms. Though mei-S332 localizes to meiotic chromosomes from prophase-I onwards, the mutant phenotype expresses very late, at a time when the sister kinetochores are functionally duplicated. It is probable that during first meiotic division, maintaining cohesion at the centromeres is not very crucial (as the sister kinetochores are not differentiated, see Goldstein[36]), and ORD can very well maintain the structural integrity of bivalents, even in absence of mei-S332, to secure proper reductional disjunction. Only when the sister kinetochores differentiate, proper functioning of mei-S332 becomes crucial to physically hold the two sisters together until anaphase-II transition. There is some evidence that ORD could promote centromeric cohesion as well[21]. However, the mechanisms of its functions at the centromere and along the chromatid arms might be different, and requires that during metaphase-I/anaphase-I transition function of ORD be preferentially removed from the chromatid arms while at the same time be retained at the centromere. It may be possible that different forms of ORD exist and their function might be mechanistically different[34]. ORD along chromatid arms might act through regulating the release of topoisomerase II inhibition, while ORD at the centromeric region might regulate functional duplication of sister kinetochores (the release of arm cohesion requires topoisomerase II, but the activity of the enzyme has been shown to be dispensable for the release of centromeric cohesion; see ref. 37).

Mutational analyses have shown that different centromeric sequences differentially affect the segregation behaviour of yeast mitotic and meiotic chromosomes. While some sequences are required for sister chromatid cohesion and faithful segregation of chromosomes during meiosis, others are important for fidelity of mitotic disjuction[38–44]. Recently, Murphy and Karpen[45] have carried out molecular and functional characterization of *Drosophila* centromere on a minichromosome, Dp1187. They have shown that besides the sequences which are necessary for minimal centromeric function, an additional ~200 kb of heterochromatic pericentromeric sequences on either side of the minimal centromere is required for faithful disjunction of chromosomes. Moreover, partial deletion of the ~200 kb repetitive sequence differentially affects segregation of chromosomes in male and female meiosis, which suggests that there are meiosis-specific functions residing in the pericentromeric heterochromatin. They have proposed a model in which the ~200 kb heterochromatic sequences are involved in maintaining sister chromatid cohesion by binding with cohesion proteins (Figure 7 *a* in ref. 45). The model is consistent with the demonstration that heterochromatin is involved in meiotic chromosome segregation[46]. Sekelsky and Hawley[47] have suggested that the mei-S332 protein might bind to the ~200 kb pericentromeric heterochromatic sequences to exert its function. However, such a binding is yet to be demonstrated. This can be done by examining the binding of mei-S332 *in vitro* to the -AATAT- satellite which comprises the ~200 kb pericentromeric sequences. Alternatively, localization of the protein on derivatives of Dp1187 might clarify this point. Lastly, it needs to be determined whether the removal of the function of mei-S332 and

ORD requires degradation of the proteins. The abundance of PEST sequences in both the proteins has been implicated as an indication that the proteins might undergo degradation at the onset of anaphase transition, through an ubiquitin independent pathway[33,34].

The recent identification of two proteins involved in maintaining cohesion between sister chromatids promises a better understanding of some basic aspects of meiosis research, particularly, the factor(s) determining chromosome segregation and the mechanism of chiasma binding. The molecular analysis of centromere function in *Drosophila*, and the identification of ORD and mei-S332 proteins has already laid the foundation for the identification of additional protein(s) which might be involved in maintaining sister chromatid coherence.

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Potential application of RAPD and RAHM markers in genome analysis of scombroid fishes

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Total genomic DNA from 30 different specimens of Indian mackerel *Rastrelliger kanagurta* (Cuvier) and 5 king seer *Scomberomorus commerson* (Lacepede) was extracted by a simple modified phenol–chloroform protocol. PCR amplification conditions using 10-mer arbitrary primers were optimized. Primers with the same nucleotide sequences produced varying Random amplified polymorphic DNA (RAPD) patterns and those with different sequences generated different banding patterns for the same template in the two commercially important scombroid fishes. Primer OPA 07 (GAAACGGGTG) yielded more polymorphic loci with high reproducibility. Better performance of the primers from Kit A (Operon Technologies, Inc) is attributed to their high G + C content. RAPDs were highly sensitive to the concentrations of primer, $MgCl_2$ and the brand of *Taq* DNA polymerase enzyme. Random amplified hybridization microsatellites (RAHM) can overcome the inherent shortcomings of RAPDs.

Random amplified polymorphic DNA (RAPD) is a novel technique of revealing DNA-based arbitrarily primed polymorphisms[1,2]. Compared with multilocus fingerprinting using microsatellite probes, the RAPD markers are more useful in discriminating populations since they are less variable over generations[3]. RAPD was found to be technically easier with low statistical error, whereas multilocus fingerprinting with 33.15 probe detected more genetic differentiation among strains of *Oreochromis niloticus*[4].

RAPDs have been particularly useful in the genetic fingerprinting of plants[5]. They are used for creating linkage maps, locating disease resistance genes and for identifying chromosome-specific markers. This PCR-based fingerprinting has begun to find application in fish and shellfish genetic analyses. RAPDs were used to detect radiation-induced DNA damages in *Oryzias latipes*[6]. Fingerprinting of 12 species of fishes using arbitrary primer PCR was reported[7]. The technique was applied for species and subspecies identification of *Oreochromis*[8]. Seven hundred and twenty one RAPD polymorphisms were identified between two laboratory strains of zebra fish, *Danio*[9] and 401 of them were used to construct a genetic linkage map[10]. Genetic variations in samples of seabass, *Dicentrarchus labrax* before and after acclimation to freshwater were investigated[11]. Inheritance of RAPD markers in the F_1 progeny of single-pair crosses between two guppy (*Poecilia reticulata*) varieties was studied[12]. Three polymorphic markers were observed out of a total of 48 RAPD loci amplified by 14 arbitrary primers in penaeid prawn *Penaeus monodon*[13].

Random amplified hybridization microsatellites (RAHM) is a new system for microsatellite DNA detection and isolation which combines RAPD amplification and oligonucleotide screening[14]. A similar technique known as *R*andom *A*mplified *M*icrosatellite *PO*lymorphisms (RAMPO) is described[15]. The rapid use and acceptance of microsatellites as genetic markers is due primarily to their comparative ease of assay via PCR

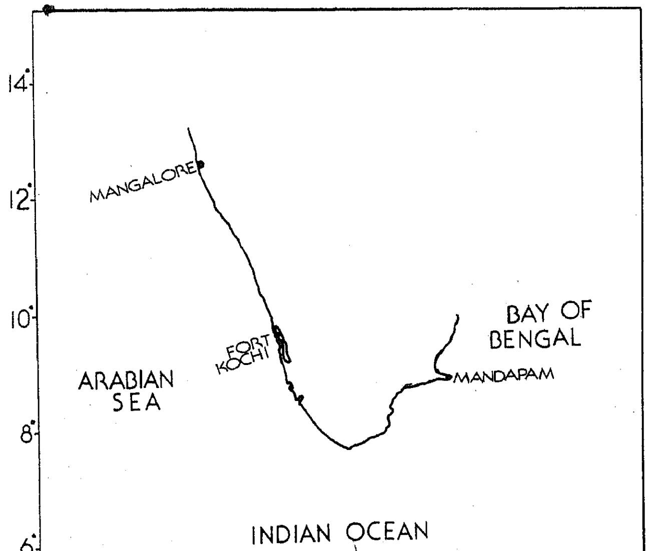

Figure 1. Map showing sampling localities.

Kit A OPA 01 (CAGGCCCTTC), OPA 02 (TGCCGAGCTC)
OPA 03 (AGTCAGCCAC), OPA 04 (AATCGGGCTG), OPA 06 (GGTCCCTGAC)
OPA 07 (GAAACGGGTG), OPA 08 (GTGACGTAGG), OPA 09 (GGGTAACGCC)
OPA 10 (GTGATCGCAG), OPA 11 (CAATCGCCGT), OPA 12 (TCGGCGATAG)
OPA 13 (CAGCACCCAC), OPA 14 (TCTGTGCTGG), OPA 15 (TTCCGAACCC)
OPA 16 (AGCCAGCGAA), OPA 18 (AGGTGACCGT), OPA 19 (CAAACGTCGG)
and OPA 20 (GTTGCGATCC).

Kit F: OPF 01 (ACGGATCCTG), OPF 02 (GAGGATCCCT)
OPF 03 (CCTGATCACC), OPF 04 (GGTGATCAGG), OPF 06 (GGGAATTCGG)
OPF 07 (CCGATATCCC), OPF 08 (GGGATATCGG), OPF 09 (CCAAGCTTTC)
OPF 10 (GGAAGCTTGG), OPF 13 (GGCTGCAGAA), OPF 18 (TTCCCGGGTT)
OPF 19 (CCTCTAGACC) and OPF 20 (GGTCTAGAGG).

Kit G: OPG 05 (CTGAGACGGA), OPG 13 (CTCTCCGCCA)
OPG 18 (GGCTCATGTG) and OPG 20 (TCTCCCTCAG).

Box 1.

and accuracy of scoring allelic types[16]. In the present study, we have optimized conditions for generating RAPD markers of two commercially important scombroid fishes and attempted RAHM technique potentially useful to discriminate fish populations. DNA samples from 30 different specimens of Indian mackerel *Rastrelliger kanagurta* (Cuvier) and 5 specimens of king seer *Scomberomorus commerson* (Lacepede) were analysed.

Materials and methods

Fish and tissue sampling

Specimens of Indian mackerel of total length range 140–250 mm were collected from commercial gill net landings at Mandapam, Mangalore and Fort Kochi and those of king seer from Mandapam during 1995–96 (Figure 1).

At Mandapam, either liver or muscle was dissected out from the fishes soon after their landings and transported to the laboratory in one of the three following media: (a) crushed ice, (b) lysate buffer (1 M Tris HCl pH 8.0; 0.4 M EDTA pH 8.0; 2% SDS), (c) 95% ethanol. Muscle samples of Indian mackerel from Fort Kochi and Mangalore fish landing centres were preserved and transported in 95% ethanol. In the laboratory all tissues were immediately stored at –70°C until DNA extraction. Total genomic DNA was extracted from 11, 11 and 8 different individual mackerels from Mandapam, Mangalore and Fort Kochi, respectively and from 5 specimens of king seer landed at Mandapam.

Extraction of genomic DNA

We have developed a simple and efficient protocol to extract total genomic DNA from field-collected fish. The preparation was found suitable equally for PCR and hybridization-based fingerprinting applications. Briefly, 0.4–1.0 g of tissue is placed in a mortar and kept at –70°C for 1–2 h. Use of liquid nitrogen to flash freeze tissue is obviated in our protocol. The frozen tissue is well ground and immediately mixed with homogenizing buffer (100 mM Tris HCl pH 8.0; 10 mM EDTA pH 8.0; 1.4 M NaCl; 1% CTAB; 0.2% mercaptethanol) and incubated with 150 μg/ml proteinase K (Sigma) at 37°C for 4–12 h. This is followed by phenol–chloroform extraction, precipitation of DNA with cold absolute alcohol, vacuum drying and resuspension in TE. RNA is removed by RNase (Sigma) at 250 μg/ml. DNA is then reextracted and resuspended in TE. Quality and quantity of the preparation were checked by agarose gel electrophoresis and spectrophotometry.

RAPD primers

A total of 35 arbitrary primers from A, F and G kits of Operon Technologies, Inc were tried to amplify DNA by PCR. The nucleotide sequences of the primers used in the present work are given in Box 1.

Optimization of primer and $MgCl_2$ concentrations

Initially four different concentrations each of arbitrary primer (0.32, 0.64, 1.28 and 2.56 μM) and $MgCl_2$ (2.0, 2.2, 2.4 and 2.6 mM) were tested to determine the optimum levels of their requirements for DNA amplification. Better resolution was obtained at 0.64 μM of primer and 2.4 mM of $MgCl_2$.

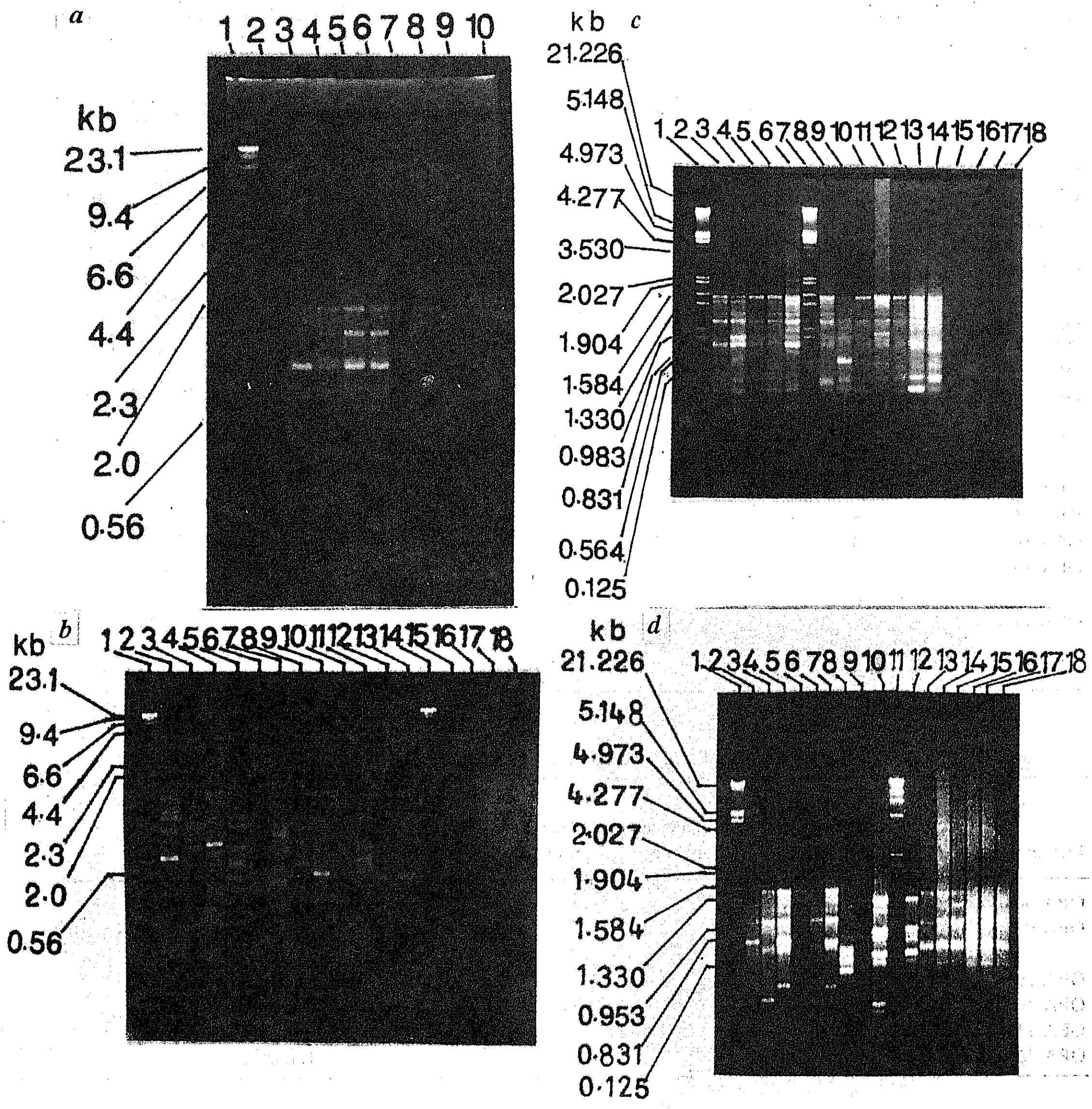

Figure 2. *a*, Effect of varying concentrations of $MgCl_2$ on RAPD patterns in Indian mackerel (*Taq* 1). Lane 1, λ *Hin*dIII; Lanes 2 and 7–10, Blank; Lane 3, 2.0 μm; Lane 4, 2.2 μm; Lane 5, 2.4 μm; Lane 6, 2.6 μm. *b*, RAPDs with OPA, OPF and OPG primers (*Taq* 1). Lanes 1 and 14, λ *Hin*dIII; Lanes 2 and 3, Indian mackerel and king seer DNAs (OPF 01); Lanes 4 and 5, Indian mackerel and king seer DNAs (OPA 07); Lanes 6, 8, 10 and 12, one Indian mackerel specimen (OPG 05, 13, 18 and 20, respectively); Lanes 7, 9, 11 and 13, one king seer specimen (OPG 05, 13, 18 and 20, respectively); Lanes 15–18, Blank. *c*, RAPDs in Indian mackerel generated by OPA 07 (*Taq* 1). Lanes 1 and 7, λ *Hin*dIII + *Eco*RI; Lanes 2 and 3, two specimens from Mandapam; Lanes 4–6 and 8–14, ten specimens from Fort Kochi; Lanes 15–18, Blank, *d*, RAPDs in Indian mackerel generated by OPA and OPF primers (*Taq* 1 except for lanes 16–18). Lanes 1 and 11, λ *Hin*dIII + *Eco*RI; Lanes 2, 3 and 5–7, five specimens from Mandapam (OPA 07); Lanes 8 and 9, two specimens from Fort Kochi (OPA 08); Lanes 13–15, two specimens from Fort Kochi and one from Mandapam (OPF 01); Lanes 16–18, same as 13–15, but used a *Taq* DNA polymerase prepared in our laboratory. The individual DNAs amplified with OPA 07 are different from those amplified with the same primer in Figure 4.

DNA amplification by PCR

One to two hundred nanograms of DNA samples were amplified in 25 μl reaction mixtures containing 10 mM Tris HCl pH 8.3, 50 mM KCl, 0.001% gelatin, 2.4 mM $MgCl_2$, 0.03 mM each of dATP, dTTP, dGTP and dCTP, 0.64 μM random primer and 1U *Taq* DNA polymerase (Amresco and Rama Biotechnologies). A total of 23 different Indian mackerel DNA templates from three locations under study were amplified with Amresco *Taq* polymerase (hereafter referred to as *Taq* 1) while a total of 30 templates (including the earlier 23) were amplified with Rama Biotechnologies *Taq* (hereafter referred to as *Taq* 2). To test the effect of primer sequences, random selection was made from the pool of DNA samples. King seer DNA was amplified

Table 1. Percentage of templates amplified by OPA-series primers (*Taq* DNA polymerase-Amresco)

Primer	No. of individual templates tested	No. of templates amplified (at least 1 polymorphic band present)	%	Remarks
OPA 01	5	0	0	
OPA 02	5	2	40.0	Bands unscorable
OPA 03	3	2	66.7	Bands unscorable
OPA 04	3	1	33.3	
OPA 06	3	3	100.0	Several scorable bands
OPA 07	23	14	60.9	Several polymorphic bands High reproducibility
OPA 08	9	2	22.2	
OPA 09	3	3	100.0	Several scorable bands
OPA 10	3	3	100.0	Several scorable bands
OPA 11	3	3	100.0	Only a single band scorable
OPA 12	3	0	0	
OPA 13	5	1	20.0	Bands unscorable
OPA 14	3	1	33.3	
OPA 15	3	0	0	
OPA 16	3	3	100.0	Few polymorphic bands
OPA 18	3	3	100.0	One template had a single scorable band
OPA 19	9	4	44.4	Two templates have only a single scorable band each
OPA 20	3	1	33.3	Only a single scorable band

Table 2. Percentage of templates amplified by OPA-series primers (*Taq* DNA polymerase-Rama Biotechnologies)

Primer	No. of individual templates tested	No. of templates amplified (at least 1 polymorphic band present)	%	Remarks
OPA 06	3	3	100.0	Several scorable bands
OPA 07	30	23	76.7	Several scorable bands which are reproducible
OPA 09	3	1	33.3	Several scorable bands
OPA 10	3	2	66.7	1-4 markers
OPA 11	3	2	66.7	2 markers
OPA 16	3	2	66.7	1-4 markers

with *Taq* 1 only. The total reaction mixture was overlaid with 25 μl of mineral oil (Sigma).

Amplification was performed in Coy II Tempcycler programmed for one initial cycle of 30 S denaturation (94°C), 30 S annealing (36°C) and 1 min extension (72°C) followed by 45 cycles of 30 S denaturation (94°C), 30 S annealing (36°C) and 2 min extension (72°C). At the end, a final extension for 7 min was performed at 72°C. Amplified products were resolved in 1.4% agarose (Sigma) gel electrophoresis in 1 × TAE. They were stained in ethidium bromide solution and under UV illumination either polaroid photographs or gel documentation pictures were taken. The RAPD bands are referred to as loci and markers interchangingly.

RAHM

RAPDs generated by OPA 07 in Indian mackerel were transferred to nylon membrane (Hybond-N, Amersham) by vacuum blotting. Thirty pmols of $(GT)_8$ (Rama Biotechnologies) were 5′ end labelled with 50 μCi of γ ^{32}PdATP. The unincorporated nucleotides were separated from the probe using Nuc Trap probe purification push column as per manufacturer's (Strategene) instructions. The purified probe had a radioactivity of 2.4×10^6 cpm. The nylon membrane containing immobilized RAPDs was prehybridized at 45°C for 3 h in a solution containing 0.1% SDS, 5 × each of SSPE and Denhardt's solution and approximately 64 μg of sonicated *E. coli* DNA. This was followed by hybridization at 45°C for 12 h in a solution containing 0.1% SDS, 5 × each of SSPE and Denhardt's solution, 64 μg of sonicated *E. coli* DNA, 1.5 g of Dextran sulphate and 120 μl of purified probe. The filter was given three final washes each for 20 min at 37°C in 3 × SSC and 0.3% SDS, dried, wrapped in cling film and developed in a phosphorimager (Molecular Dynamics Inc).

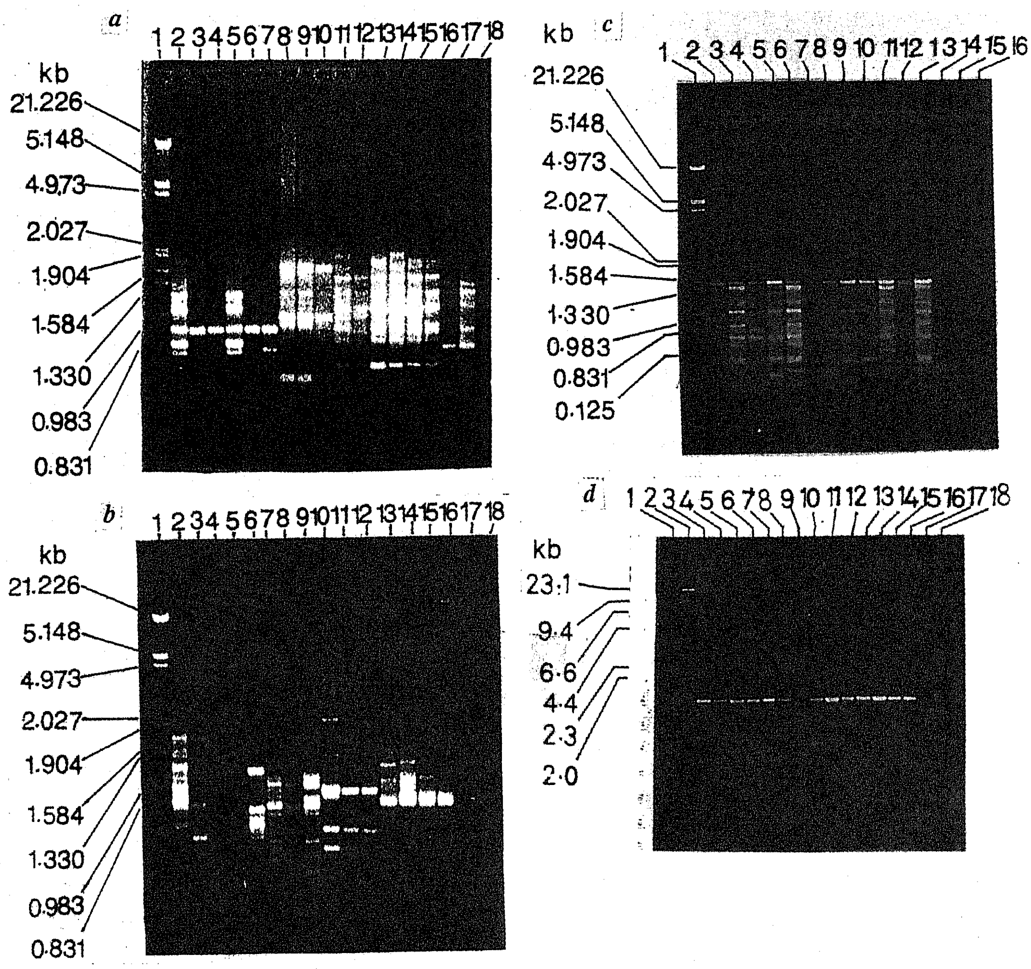

Figure 3. ***a***, RAPDs in Indian mackerel generated by OPA and OPF primers (*Taq* 1), Lane 1, λ *Hin*dIII + *Eco*RI; Lanes 2, 5, 12, 15 and 18, a specimen from Fort Kochi (OPA 18, 19, 06, 10 and OPF 04, respectively); Lanes 3, 6, 11, 14 and 17, a specimen from Mandapam (OPA 18, 19, 06, 10 OPF 04, respectively); Lanes 4 and 7, a specimen from Mangalore (OPA 18 and 19, respectively); Lanes 8–10, 13 and 16, a specimen from Mangalore (OPF 18, 19, 20, OPA 06 and 10, respectively). ***b***, RAPDs in Indian mackerel generated by OPA and OPF primers (*Taq* 1). Lane 1, λ *Hin*dIII + *Eco*RI; Lanes 2, 7, 10, 13 and 14, a specimen from Mandapam (OPF 03, OPA 09, 11, 14 and 16, respectively); Lanes 3, 5, 6, 9, 12 and 16, a specimen from Mangalore (OPF 06, 09, OPA 04, 09, 11 and 16, respectively); Lanes 4, 8, 11 and 15, a specimen from Fort Kochi (OPF 09, OPA 09, 11 and 16, respectively); Lanes 17 and 18, Blank. ***c***, RAPDs in Indian mackerel generated by OPA 07 (*Taq* 1). Lane 1, λ *Hin*dIII + *Eco*RI; Lanes 2 and 11–13, four specimens from Fort Kochi; Lanes 5, 7, 9 and 10, four specimens from Mangalore; Lanes 14–16, Blank. The individuals are different from ones whose DNAs were amplified with OPA 07 as in Figure 2 *c*, *d*. ***d***, RAPDs in Indian mackerel generated by OPA 07 (*Taq* 2). Lanes 1 and 2, λ *Hin*dIII; Lanes 3–7, five specimens from Mandapam; Lanes 8–11, four specimens from Fort Kochi; Lanes 12–16, five specimens from Mangalore. Lanes 17–18, Blank.

Results

Relatively better resolution of RAPD loci was obtained when 2.4 mM $MgCl_2$ was used (Figure 2 *a*, lane 5). With random primers from Kit G, 100 ng of genomic DNA each from king seer and Indian mackerel were amplified. While comparing lanes 6, 8, 10 and 12 with 7, 9, 11 and 13 in Figure 2 *b* it is apparent that the OPG random primers amplify DNA of king seer better than that of Indian mackerel. Further, lanes 2–13 show that difference in primer sequence would produce different RAPD patterns for the same template in both these scombroid fishes.

Considering the large number of templates tested, high percentage of amplification (amplification refers to the presence of at least one polymorphic band per gel) as well as better reproducibility, OPA 07 was found to be the ideal choice for producing RAPDs in Indian mackerel (Table 1). Figures 2 *c*, *d* and 3 *a–c* show RAPDs using *Taq* 1.

Performance of OPA 07 was even better in amplification of Indian mackerel DNA with *Taq* 2 (Table 2). Number as well as patterns of markers for the same templates generated with the same primers and two *Taq* enzymes were different. Figures 3 *d*, 4 *a–c* depict RAPD patterns using *Taq* 2.

Table 3. Percentage of templates amplified by OPF-series primers (*Taq* DNA polymerase-Amresco)

Primer	No. of individual templates tested	No. of templates amplified (at least 1 polymorphic band present)	%	Remarks
OPF 01	5	3	60.0	
OPF 02	8	2	25.0	
OPF 03	3	1	33.3	
OPF 04	3	2	66.7	One template had a single scorable band
OPF 06	3	1	33.3	
OPF 07	3	1	33.3	Bands unscorable
OPF 08	3	1	33.3	Bands unscorable
OPF 09	3	2	66.7	One template had a single scorable band
OPF 10	3	0	0	
OPF 13	3	2	66.7	One template had a single band Others had all unscorable bands
OPF 18	3	2	66.7	One template had a single scorable band
OPF 19	3	1	33.3	
OPF 20	3	1	33.3	

None of the random primers from Kit F produced loci in 100% of templates tested (Table 3). Further, the number of loci was less and reproducibility low. Figures 2 *d*, 3 *a* and *b* depict RAPD markers in different specimens of Indian mackerel sampled from Mandapam, Mangalore and Fort Kochi amplified with OPF primers.

RAHM markers of Indian mackerel are shown in Figure 4 *d*. Lanes 3, 4, 7, 8, 10 and 13 contain a total of 9 bands having complementary sequence of $(GT)_8$. This gives 25% of positive fragments after hybridization with the dinucleotide probe. Signal intensity of bands depends both on the microsatellite motifs and the abundance of the particular loci.

Discussion

RAPD could prove to be a useful tool to estimate genetic variability, relatedness, inbreeding levels, species/strain verification, pedigree analysis, detection of economic traits and in other marker-based studies in fish and shellfish. RAPDs that are diagnostic at different taxonomic levels can be generated by employing different primers and they can be used to determine the relatedness between taxa for which diagnostic RAPD fingerprints have been established[3]. However, for this PCR-based fingerprinting to provide supporting evidence for taxonomic relationships in fishes, data should be generated using adequate sample size for each species[7]. RAPD loci produced by the same random primer showed markedly different patterns between Indian mackerel and king seer in our work. Similarly, arbitrary primers of different sequences yielded different banding patterns in the same template of both scombroid fishes. This indicates utility of RAPDs by manipulation of primer sequence to generate amplification products of desired complexity to suit different purposes like genetic mapping or genotyping[7].

In a work on RAPD analysis of 12 species of fishes, there was no clear relation observed between the length of random primers and the number of fragments amplified. However, there were relatively more amplified products with primers having higher G + C content[7]. The number of products may be related to the G + C content of primers and template DNA sequence rather than to the primer length[17]. We have tested primers having G + C content 60–70% (Kit A), 50–60% (Kit F) and 50–70% (Kit G). Better performance of primers from Kit A in terms of greater percentage of templates amplified and more number of loci generated, could be attributed to their higher G + C content.

Our study clearly shows high sensitivity of RAPDs to PCR conditions. For example, the same DNA templates amplified with two different commercial brands of *Taq* DNA polymerases generated different fingerprints. This is in conformity with earlier observations[5]. Concentrations of primer and $MgCl_2$ were critical *vis-à-vis* resolution and reproducibility of RAPD loci. However, we did not find the DNA template concentration to be a decisive factor within a wide range of 1–200 ng. Total genomic DNA extracted from frozen, buffered and frozen or alcohol-preserved and frozen tissues yielded uniform results in RAPD-PCR.

Relative ease of development, low expense and potential usefulness for any kind of organism make RAPDs attractive molecular markers. However, criticisms are levelled at them for their genetic dominance (inability to differentiate homozygotes from heterozygotes), low degree of polymorphisms and exquisite sensitivity to amplification conditions. Hybridizing RAPDs with

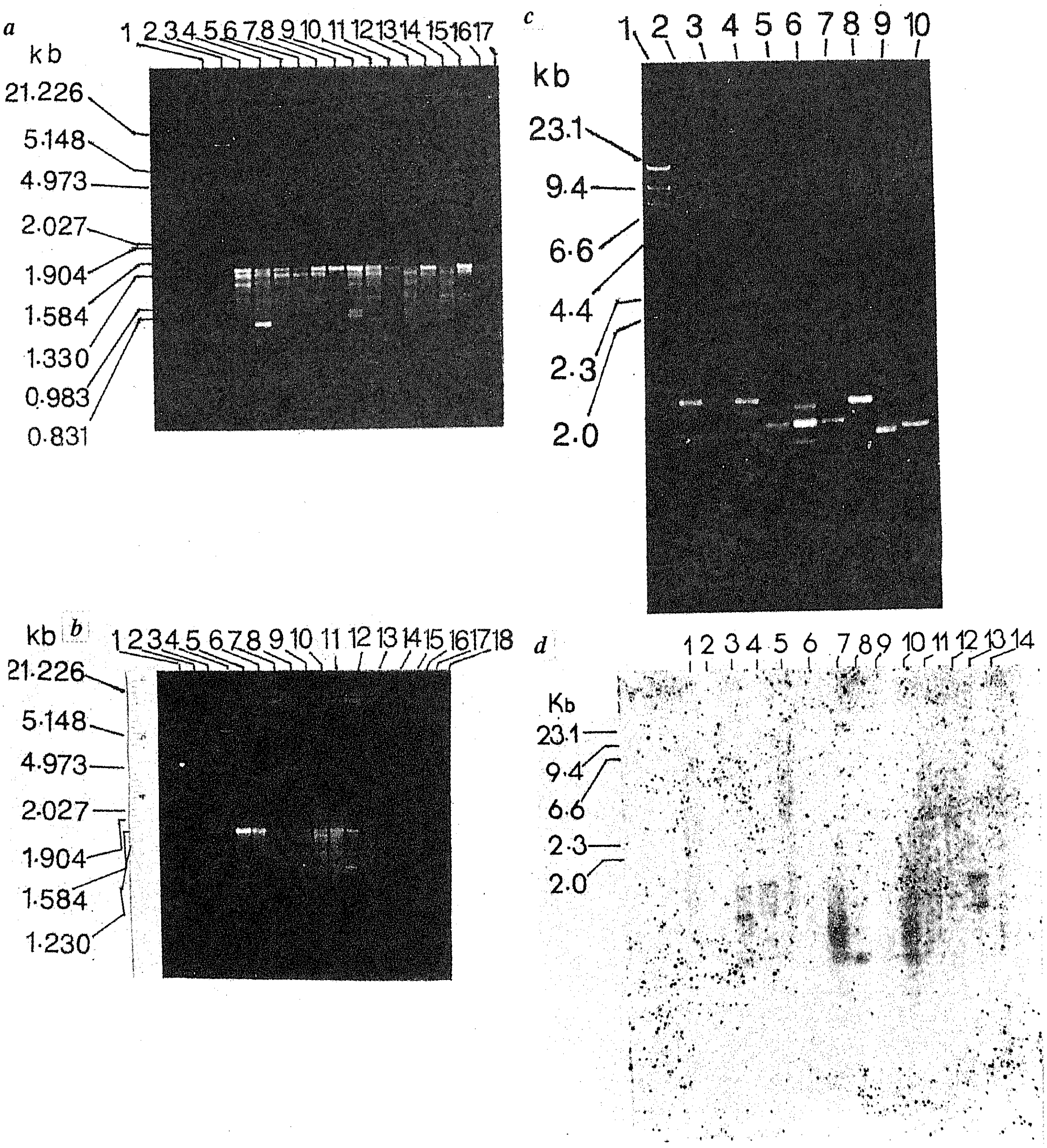

Figure 4. *a*, RAPDs in Indian mackerel generated by OPA 07 (*Taq* 2). Lanes 1, 16 and 17, Blank; Lane 2, λ *Hin*dIII; Lanes 3, 9, 10 and 15, four specimens from Mangalore; Lanes 4, 5, 8 and 11, four specimens from Fort Kochi; Lanes 6, 7 and 12–14, five specimens from Mandapam. The individual DNAs depicted here are different from those in Figure 3 *d*. *b*, RAPDs in Indian mackerel generated by OPA 07 (*Taq* 2). Lanes 1–3 and 13–18, Blank; Lane 4, λ *Hin*dIII + *Eco*RI; Lanes 5 and 12, two specimens from Mandapam, Lanes 6, 9 and 10, three specimens from Mangalore; Lanes 7, 8 and 11, three specimens from Fort Kochi. All individual DNAs depicted here are different from those in Figures 3 *d* and 4 *a*. *c*, RAPDs in Indian mackerel generated by OPA series primers (*Taq* 2). Lane 1, λ *Hin*dIII + *Eco*RI; Lanes 2–4, three specimens from Mandapam (OPA 06); Lanes 5 and 6, two specimens from Mandapam (OPA 10); Lanes 7 and 8, two Mandapam specimens (OPA 11); Lanes 9 and 10, two Mandapam specimens (OPA 16). *d*, RAPDs depicted in Figure 3*d* southern-blotted and hybridized with 5′ end labelled $(GT)_8$. RAPD loci of less than 2 kb are seen hybridized in lanes 3, 4, 7, 8, 10 and 13.

microsatellite probes can yield novel and highly reproducible fingerprinting profiles. Ethidium bromide staining can detect only the most abundant amplicons whereas majority of minor fragments may remain below the detection level to form a background smear in the gel. The ubiquitous presence of dinucleotide repeats in eukaryotic genomes provides a means of visualizing a subset of such minor amplification products by hybridization[14].

Comparing lanes 13 and 3 of Figure 4 *d* respectively with 15 and 5 of Figure 3 *d* in our paper shows generation of more number of bands when RAPDs were southern-blotted. RAHM technique can provide more information from RAPD gels and also help to reveal microsatellite genomic clones without the time-consuming screening of genomic libraries[13]. This technique offers immense potential for genome analyses in fish and shellfish.

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The Rayleigh–Taylor instability of a viscous fluid layer with viscosity stratification

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The effects of viscosity stratification and surface tension on Rayleigh–Taylor (RT) instability in a finite thickness layer of an incompressible viscous fluid bounded above by a denser fluid and below by a rigid impermeable surface have been studied using linear stability analysis. A relation for the RT-instability growth rate is found by calculating the eigenvalues of the stability equation. It is shown that the shape of the dispersion curve is controlled by the ratio of surface tension to the stress gradient, with the layer thickness and the viscosity stratification affecting the rate of growth of the instability. The growth rate, infact, is shown to increase with increase in viscosity stratification and the thickness of the fluid layer. The results are shown to revert to those of the case of a viscous fluid layer in the absence of viscosity stratification.

THE study of instability of the interfaces is of great importance in view of its fundamental role in understanding of the control and exploitation of many of the basic physical, chemical and biological process. The interface in some of these instances occurs due to the superposition of two fluids of different densities. The instability of the interface occurring under gravity when a heavier fluid is supported by a lighter one or equivalently when a lighter fluid is accelerated towards the heavier fluid is widely known as the Rayleigh–Taylor (RT) instability[1]. These instabilities have been the subject of considerable interest in recent years mainly because of their frequent encounter in nature and in many practical applications[2,3]. They also occur in ocean tides, the accelerated interstellar clouds driven by new born stars, supernova events, sinking of slabs of tectonic plates, etc. The analysis of motion induced by the instability of the highly viscous layers in the earth's interior indeed provide rational models for the prediction of continental drifts and the volcanic activities. These instabilities are further known to occur in thin aqueous layers of biological fluids which form an integral part of the various organs and their stability plays an important role in the organ functions. In technological contexts, the RT-instabilities occur in the inertial confinement fusion (ICF) which is based on the implosion of thin deuterium (D)–tritium (T) targets driven by laser or ion beams. The target during the implosion process could be hydrodynamically unstable subject to these instabilities, which destroy the symmetry of compression of the D–T fuel preventing the high density and temperature required for thermonuclear burning with high gain in energy. These instabilities are considered responsible, in material processing applications, for grain boundary failure in metals and cracking of cross-linked polymers. The polymer failure, infact, has been shown to occur often by the formation and growth of planar defects known as crazes; both craze tip advance and craze widening are attributed to these instabilities. These instabilities are responsible for development of non-uniformities that lead to streaked and mottled layers in the growth of thin films in a variety of device fabrication applications. Thus there is evidently a considerable current interest in studying the dynamics of fluid layers from both fundamental and technological perspectives. On the fundamental level the study of layer dynamics concerns a more general problem of evolution of active and passive interfaces; the issues of interest being the formation of ordered interfacial structures and the long time asymptotic behaviour of the deformed interfaces. The understanding and controlling of the deformation of viscous layers, from the technological point of view, provides useful information for suppressing or eliminating these hydrodynamic instabilities. It is well known that various physical factors such as surface tension, viscosity, density gradients and shear rates in flowing films greatly influence the development of RT-instability. Brown[4] has recently investigated the linear instability of a highly viscous fluid layer of finite thickness and shown that such a film if stagnant would rupture as a consequence of this instability. Further, Newhouse and Pozrikidis[5] have studied the nonlinear viscous instability of a liquid layer as a function of surface tension and the ratio of viscosities of the two fluids and have shown that for moderate surface tension, the instability of the layer leads to the formation of periodic array of viscous plumes which penetrate into the overlaying fluid layers. However, the combined effect of surface tension and viscosity variation on RT-instability has not been given much attention in spite of its importance in many practical situations.

The motivation of the present study is to extend the work of Brown[4] to include the effect of viscosity stratification to know its influence on the RT-instability in a thin layer of an incompressible viscous fluid confined above by an interface with a denser fluid and below by a rigid impermeable boundary by using linear perturbation analysis. The formalism developed in the present work is primarily to elucidate the influence of the combined effects of stress gradient, surface tension, viscosity stratification and the layer thickness on the RT-instability. We found that the viscosity stratification

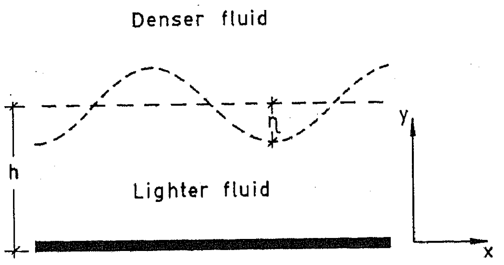

Figure 1. Physical configuration.

affects only the nature of the growth of the instability and does not change the shape of the dispersion curve.

We consider the case of two incompressible fluids with equal viscosities $\mu_F = \mu_L = \mu$ and densities ρ_L and ρ_F, where suffixes F and L refer to lower fluid (film) and upper denser fluid respectively as shown in Figure 1. The interface is characterized by surface tension γ and is governed by the layer thickness h and the interface elevation η. The flow within the viscous layer and the overlying fluid in the absence of any external force is governed by the following equations.

$$\rho\partial u_i/\partial t + \rho u_j(\partial u_i/\partial x_j) = -\partial p/\partial x_i + \partial/\partial x_j \{\mu(\partial u_i/\partial x_j + \partial u_j/\partial x_i)\} \qquad (1)$$

and

$$\partial u_j/\partial x_j = 0, \qquad (2)$$

where ρ is fluid density, u_i, u_j are velocity components, p the pressure and μ the coefficient of viscosity. The following assumptions[6] enabled us to use the creeping flow approximation:

(i) The film thickness h, is much smaller than the thickness H, of the denser fluid above the film. That is

$$h << H. \qquad (3)$$

(ii) The Strouhal number ε, which is a measure of the local acceleration to inertial acceleration in equation (1) is small. That is

$$\varepsilon = \frac{L}{TU} \ll 1 \qquad (4)$$

Here $U = \nu/L$ is the characteristic velocity, $\nu = \mu/\rho_f$ is the kinematic viscosity, $L = \sqrt{\gamma/\delta}$ is the characteristic length, $\delta = g(\rho_L - \rho_F)$, γ is the surface tension, $T = \mu\gamma/h^3\delta^2$ is the characteristic time scale. This assumption enabled us to neglect local acceleration term $(\partial u_i/\partial t)$ in eq. (2).

(iii) We consider high viscous fluid so that inertial acceleration term in eq. (2) can be neglected in comparison with viscous term.

(iv) The interface elevation η is assumed to be small compared with the film thickness h. That is,

$$\eta/h << 1. \qquad (5)$$

These assumptions enabled us to use the creeping flow approximations which allow us to neglect certain terms in the perturbation problem in order to arrive at closed form asymptotic equation for the interface evolution. In other words, we confine to the conditions of creeping flow in two dimensions and hence ignore the inertial terms. We superimpose on the basic static state of constant pressure a small symmetrical perturbation in both the velocity and pressure fields to obtain after linearizing, the following equations for the perturbed fields.

$$\frac{\partial p}{\partial x} = \mu\left(\frac{\partial^2 u}{\partial x^2} + \frac{\partial^2 u}{\partial y^2}\right) + \frac{\partial \bar{\mu}}{\partial y}\left(\frac{\partial u}{\partial y} + \frac{\partial v}{\partial x}\right), \qquad (6)$$

$$\frac{\partial p}{\partial y} = \mu\left(\frac{\partial^2 v}{\partial y^2} + \frac{\partial^2 v}{\partial x^2}\right) + 2\left(\frac{\partial \bar{\mu}}{\partial y}\right)\left(\frac{\partial v}{\partial y}\right), \qquad (7)$$

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0, \qquad (8)$$

where u and v are the velocity components in x and y directions respectively and $\bar{\mu}$ is the mean viscosity which is a function of y only. The problem of concern is the growth rate of a small periodic perturbation of the interface. We therefore assume a variation in the velocity components, pressure and the interface elevation along x-axis to be of the form

$$[u,\ v,\ p,\ \eta] = [u(y),\ v(y),\ p(y),\ \eta(y)]\ \exp(i\alpha x + nt), \qquad (9)$$

where α is the wave number and n, the growth rate. The eqs (3)–(5) in view of eq. (6) transform into the following equations

$$i\alpha p = \mu(-\alpha^2 u + \mathrm{D}^2 u) + \mathrm{D}\bar{\mu}\ (\mathrm{D}u + i\alpha v), \qquad (10)$$

$$\mathrm{D}p = \bar{\mu}(-\alpha^2 v + \mathrm{D}^2 v) + 2\mathrm{D}\bar{\mu}\mathrm{D}v, \qquad (11)$$

$$i\alpha u + \mathrm{D}v = 0, \qquad (12)$$

where $\mathrm{D} = \mathrm{d}/\mathrm{d}y$. We further consider a realistic mean viscosity stratification because it pertains to stable stratification, to be given by

$$\bar{\mu} = \mu_0 \exp(-\beta y), \qquad (13)$$

where μ_0 is a constant characterizing uniform viscosity and β the viscosity stratification factor. The elimination of pressure from eqs (10) and (11) and subsequent use of eqs (12) and (13) gives the following governing equation

$$\mathrm{D}^4 v - 2\beta\mathrm{D}^3 v - (2\alpha^2 - \beta^2)\mathrm{D}^2 v + 2\alpha^2\beta\mathrm{D}v + (\alpha^2\beta^2 + \alpha^4)v = 0. \qquad (14)$$

The foregoing equations have to be solved subject to the boundary and the interfacial conditions. The validity of the no-slip condition at the rigid boundary requires that not only normal component but also the horizontal component of velocity vanish on the bounding surface. This requirement in view of the mass conservation equation is ensured by the following conditions

$$v = \mathrm{D}v = 0 \text{ on the rigid surface at } y = 0. \tag{15}$$

The requirement of vanishing of the tangential stress at the free interface is

$$\frac{\partial u}{\partial y}+\frac{\partial v}{\partial x}=0.$$

This, using eq. (9), becomes $\mathrm{D}u + i\alpha v = 0$.

The required surface condition, from this using $u = (i/\alpha)\mathrm{D}v$ obtained from eq. (12), is

$$\mathrm{D}^2v + \alpha^2v = 0 \text{ on the interface at } y = h. \tag{16}$$

However, the normal stress at the interface is discontinuous and it is compensated by the surface tension. Therefore the normal stress is determined by the combined effects of stress gradient, surface tension and shear on the interface and is given by

$$p = -\delta\eta - \frac{\gamma\partial^2\eta}{\partial x^2} + \bar{\mu}\ \mathrm{D}v \text{ at } y = h. \tag{17}$$

Further the kinematic condition governing the interface is given by

$$\frac{\partial\eta}{\partial t}+u\frac{\partial\eta}{\partial x}+v\mathrm{D}\eta=0 \text{ at } y=h. \tag{18}$$

We consider the evolution of the interface which is described by the following interface equation

$$y = h - \eta. \tag{19}$$

The pressure balance eq. (17) in view of eq. (9) is given by

$$p = -\delta\eta + \alpha^2\gamma\eta + \bar{\mu}\mathrm{D}v. \tag{20}$$

Further, the kinematic condition expressed by eq. (18) in view of eq. (9) and the interface eq. (19) after linearizing yields

$$v = \frac{\partial\eta}{\partial t} \text{ at } y = h.$$

This using eq. (9) becomes

$$n = v/\eta. \tag{21}$$

The elimination of pressure from eqs (10) and (20) and subsequent use of eqs (10) and (18) lead to the following dispersion relation

$$n = (\alpha^2/\mu_0)v \exp(\beta h)(\delta - \alpha^2\gamma)/(2\alpha^2\mathrm{D}v - \mathrm{D}^3v).. \tag{22}$$

The growth rate n in the absence of the viscosity stratification ($\beta = 0$) may be seen to yield the result of that of the case of a fluid layer of constant viscosity considered by Brown[4]. We see that the shape of the entire dispersion curve is controlled by the ratio δ/γ, with the viscosity stratification factor β and the layer thickness h affecting only the rate of growth of the instability.

The roots λ_i (i = 1 to 4) of the characteristic equation

$$\lambda^4 - 2\beta\lambda^3 - 2(\alpha^2 - \beta)\lambda^2 + 2\alpha^2\beta^2\lambda + \alpha^2(\beta^2 + \alpha^2) = 0 \tag{23}$$

of the differential equation (14) are

$$\lambda_1, \lambda_2 = \frac{1}{2}(\beta \mp \Delta_1),\ \lambda_3, \lambda_4 = (\beta \mp \Delta_2)\ldots, \tag{24}$$

where $\Delta_1 = [\beta^2 + 4\alpha(\alpha + i\beta)]^{1/2}$, $\Delta_2 = [\beta^2 + 4\alpha\ (\alpha - i\beta)]^{1/2}$.

We see that for the entire range of values of α and β relevant to the present problem, eq. (24) provides a pair of complex and distinct roots except at $\alpha = 0$. The solution of eq. (14) in this context satisfying the boundary conditions expressed in eqs (15) and (16) may be given by

$$\begin{aligned} v = C[\{&\cos(b_1y) + A\sin(b_1y)\}\exp(a_1y) \\ &- \{\cos(b_2y) + B\sin(b_2y)\}\exp(a_2y)], \end{aligned} \tag{25}$$

where A, B and C are constants and a_i's and b_i's are the real and imaginary parts of the roots of eq. (24) and we have further, $A = A_1/A_2$ and $B = A(b_2/b_1) - (a_1 - a_2)/b_1$, where A_1 and A_2 are given by

$$\begin{aligned} A_1 = \Big[&\big\{(2a_2 - a_1)a_1 - b_1^2 + \alpha^2\big\}\cos(b_1h) \\ &+\big\{((a_2 - a_1)/b_1)(a_1^2 - b_1^2 + \alpha^2) - 2a_1b_1\big\} \\ &\sin(b_1h)\Big]\exp(a_1h) + \Big[\big\{(b_2^2 - a_2^2 - \alpha^2)\cos b_2h \\ &+2a_2b_2\sin b_2h\Big]\Big\}\exp(a_2h). \end{aligned} \tag{26}$$

$$\begin{aligned} A_2 = \Big[&\big\{b_2(b_1^2 - a_1^2 - \alpha^2)/b_1\big\}\sin(b_1h) - 2a_1b_2\cos(b_1h)\Big] \\ &\exp(a_1h) + \Big[(a_2^2 - b_2^2 + \alpha^2)\sin(b_2h) + 2a_2b_2\cos(b_2h)\Big] \\ &\exp(a_2h). \end{aligned} \tag{27}$$

We employ the perturbation wavelength given by $\lambda^* = (\gamma/\delta)^{1/2}$ and the growth rate given by $n^* = n\mu_0/(\delta\gamma)^{1/2}$ as characteristic scales associated with the problem to render the equations dimensionless for stability analysis. In view of these parameters and the eq. (25), the dimensionless dispersion relation after omitting the asterisks for simplicity is given by

$$n = [(1 - \alpha^2)/\alpha][\alpha^3Q/(2\alpha^2R - S)]\exp(\beta h), \tag{28}$$

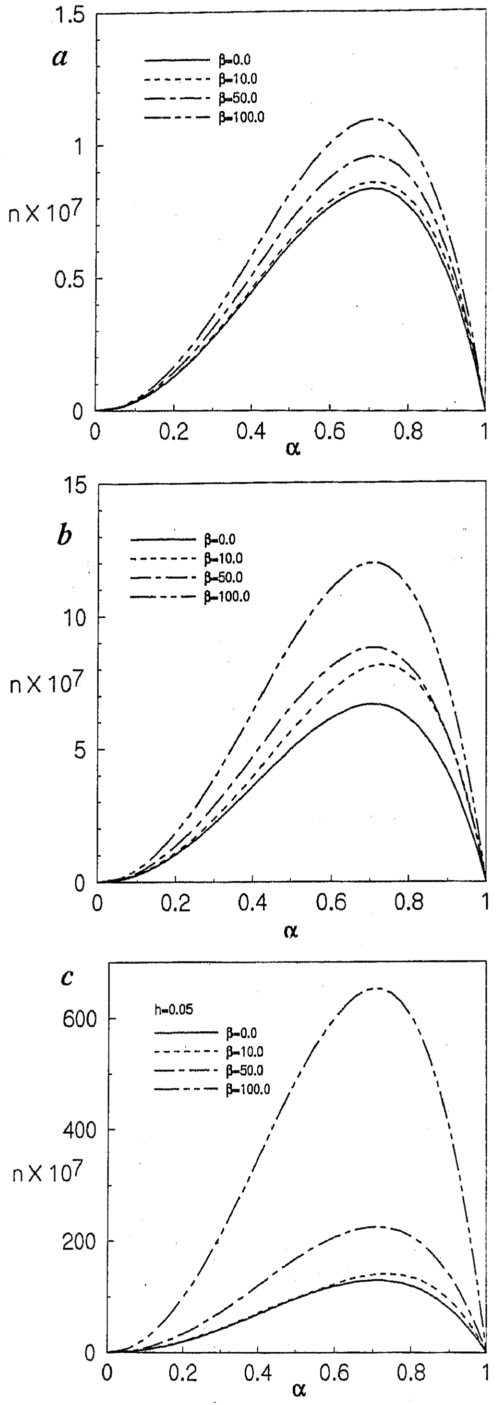

Figure 2 *a–c*. A few typical plots of the growth rate n against the wave number α.

where

$$Q = C[\{\cos(b_1h) + B\sin(b_1h)\}$$
$$\exp(a_1h) - \{\cos(b_2h) + A\sin(b_2h)\}\exp(a_2h)]. \quad (29)$$

$$R = C[\{(a_1 + Bb_1)\cos(b_1h) + (Ba_1 - b_1)\sin(b_1h)\}$$
$$\exp(a_1h) - \{(a_2 + Ab_2)\cos b_2h + (Aa_2 - b_2)$$
$$\sin(b_2h)\}\exp(a_2h)]. \quad (30)$$

$$S = C\Big[\big\{(a_1^3 - Bb_1^3 + 3a_1b_1(Ba_1 - b_1))\cos(b_1h)$$
$$+(Ba_1^3 + b_1^3 - 3a_1b_1(a_1 + Bb_1))\sin(b_1h)\big\}\exp(a_1h)$$
$$-\big\{(a_2^3 - Ab_2^3 + 3a_2b_2(Aa_2 - b_2))\cos(b_2h)$$
$$+(Aa_2^3 + b_2^3 - 3a_2b^2(a_2 + Ab_2))$$
$$\sin(b_2h)\big\}\exp(a_2h)\Big]. \quad (31)$$

A few typical plots of the growth rate n against the wave number α for a range of viscosity stratification factor β and the layer thickness h of the film are given in Figure 2.

It may be seen from Figure 2 that the whole dispersion curve is controlled by the ratio of stress gradient to surface tension, δ/γ, with the viscosity stratification factor β and the layer thickness h just affecting the nature of the growth of the instability. Eq. (28) reveals that the characteristic length L is of the order of critical wavelength and the maximum growth rate occurs at a wavelength of the same order as L. We note that our results on the growth rate in the absence of viscosity stratification ($\beta = 0$) revert to the case of Brown[4]. When $\beta \neq 0$, the frequency of oscillations of RT instability tend to amplify with increasing β in proportion to exp (βh), where the viscosity stratification $\beta = (-1/\bar{\mu})\,(\mathrm{d}\bar{\mu}/\mathrm{d}y)$ is the reciprocal of the film thickness h. This variation can readily be interpreted physically in terms of energy flux of oscillations, since it just compensates for the upward decrease of viscosity of thin film in proportion to exp $(-\beta y)$ in maintaining the flux constant. It may be observed from the plots given in Figure 2 that for given viscosity stratification factor β the RT-instability increases with increase in the layer thickness h. This behaviour of the instability growth rate may be because at higher values of the stratification factor β, the fluid viscosity $\exp(-\beta y)$ is very small at the interface and hence due to the reduced damping of the disturbance the surface becomes increasingly unstable. Initially for $\alpha < 0.7$, buoyancy dominates and every point of the interface moves faster with a velocity proportional to the elevation η of that point and elevation grows. For $\alpha > 0.7$ the surface tension action which was negligible initially becomes enhanced and elevations are reduced, the steepening process is slowed down. We note that though the values of β and h help in affecting the nature of the RT-instability growth rate, no complete change in nature of instability is possible. It is evident that although the present work is limited to the linear analysis, it throws much light on the size scale and behaviour of RT-instabilities in thin viscous layers relevant in numerous problems of natural and practical applications discussed earlier.

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Mutational study and prevalence of Duchenne/Becker muscular dystrophy in different caste groups in Uttar Pradesh

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Social relationships have implication on the incidence of genetic diseases in the population. To analyse this aspect in Duchenne/Becker muscular dystrophy (DMD/BMD) patients, DNA profile of the dystrophin locus was carried out by PCR and Southern hybridization. The retrospective analysis of clinical and molecular data of the patients showed higher prevalence of the disease in *brahmins* and *vaishyas* as compared to the other caste groups in this region. However, the mutational pattern is not very different in various caste groups. The results have been explained by presence of repetitive dA–dT or transposon-like sequences in the introns of the dystrophin gene.

DUCHENNE/Becker muscular dystrophies are allelic X-linked recessive genetic disorders with a frequency of 1 in 3500 live male births[1]. Duchenne type (DMD) is a severe form of the disease which causes loss of ambulation in early life, and death usually occurs by the end of the second decade. Becker type (BMD) disease follows a milder course and patients can remain ambulatory till 40–50 years of age[2].

Population-based variations in the incidence and pattern of gene mutations in DMD/BMD are well recognized. Higher prevalence of DMD has been reported in Indian migrants in UK as compared to local and other ethnic populations in that region[3]. In various European populations, gene mutations in DMD/BMD patients show nonrandom distribution which changes with linguistic boundaries of the continent[4]. We have also reported higher proportion of dystrophin gene deletions in the patients from North India[5]. Moreover, analysis of the carrier status of mothers of these patients by quantitative polymerase chain reaction has yielded very high frequency of new mutations[6]. Further, the prevailing caste-based division in Indian society provides a unique opportunity to study genetic consequences of the disease in the population. The present study depicts the retrospective analysis of molecular data related to gene mutations in DMD/BMD patients belonging to various caste groups of Uttar Pradesh.

All patients were enrolled in the out-patient department. Most of the patients came from Eastern and Central part of UP, but some were also from the north-western part of the state (Figure 1). The clinical diagnosis of DMD/BMD was based on detailed proforma which included age of onset, relative strength of muscles, Gower's sign, Pradhan's sign[7,8], degree of ambulation and levels of creatine phosphokinase (CPK) in serum. Multiplex PCR and Southern hybridization were carried according to standard methods[9]. Translational reading frame of the putative mRNA in deletional cases was determined by border type analysis[10]. Caste information was voluntarily provided by the guardians. Patients having parents/grandparents with intercaste marriages were excluded from the present analysis.

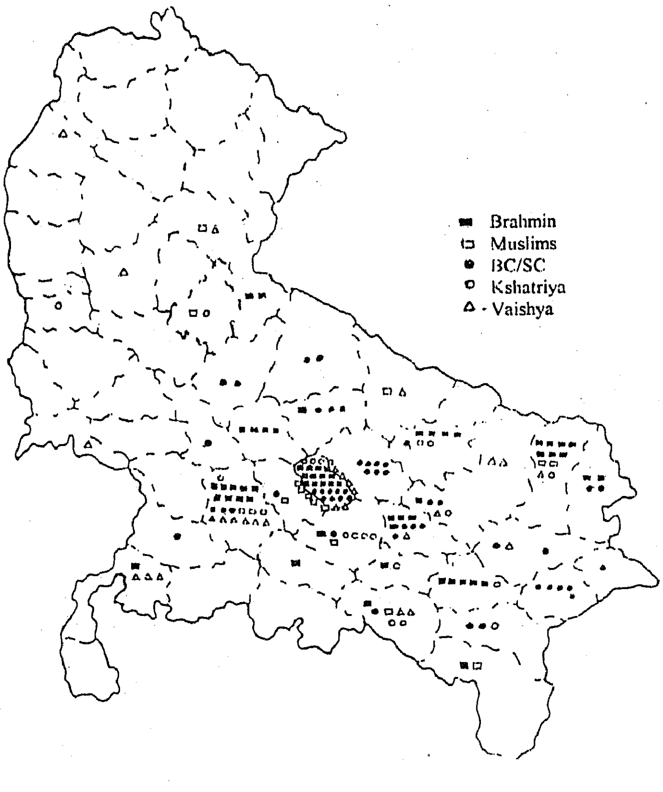

Figure 1. Regional distribution of cases of DMD/BMD among different caste groups in Uttar Pradesh.

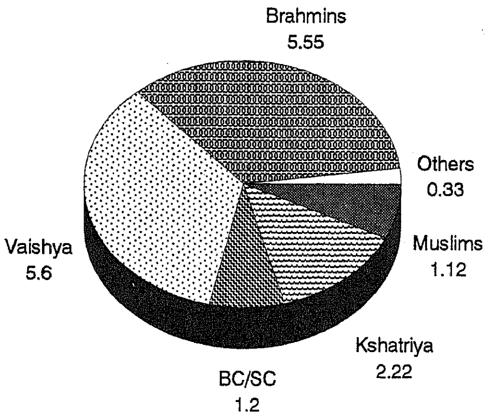

Figure 2. Comparative incidence of disease in different caste groups in UP. Relative figures are obtained by dividing number of patients with per cent population of particular caste.

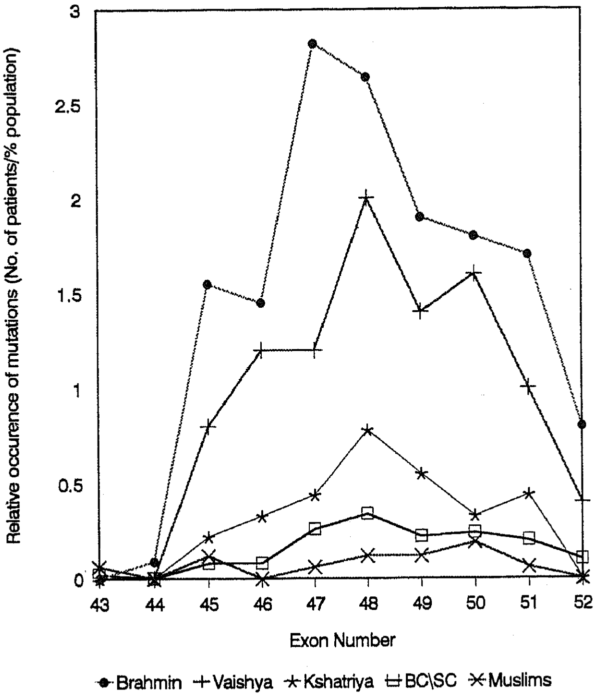

Figure 3. Relative involvement of central region exons of dystrophin gene mutations among different caste groups.

Retrospective analysis of clinical and molecular data on the basis of major caste groups of the region showed that more patients belong to *brahmins* as compared to other caste groups. Supposing that the number of patients registered in the present study is proportional to their number in general population of the region, relative incidence of disease was calculated by dividing number of patients to their percentage population in UP (based on data from *India Today*, 15 May 1996). The relative incidence of DMD/BMD was found to be much higher in *brahmins* and *vaishyas* as compared to other caste groups (Figure 2). From combined results of PCR and Southern hybridization, intragenic deletions could be detected in 70% of cases with DMD/BMD. The proportion and over-all pattern of deletions in different caste groups were similar, though minor variations in exonic involvements were observed at the central hot spot region of the gene (Figure 3).

The biology of social relation of humans is most interesting and debatable. The social structure of a community has its impact on the inheritance of genetic diseases. Communities with consanguineous marriages show higher incidence of some genetic diseases. It is impossible to plan selective breeding in human population, but in India, prevailing caste-based marriage systems provide some control over random breeding. The people generally prefer marriages in their own caste and the effect of this controlled breeding may be studied by analysing occurrence of genetic defects and polymorphism in different caste groups[11,12].

Higher relative incidence of DMD/BMD has been observed in *brahmins* and *vaishyas* as compared to other caste groups. It may be attributed to some degree of endogamous marriages followed in these caste groups. Both castes have various subgroups which prefer marriages in their own groups. Though other castes also have subgroups, the populations of *brahmins* and *vaishyas* are comparatively low in the region (*India Today*, May 15 1996), which limits their gene pool.

In DMD/BMD, about two-thirds of the patients show intragenic deletions ranging from one to several exons and the remaining one third patients are believed to have point mutations and other regulatory defects[13]. We had earlier observed relatively higher proportion of gene deletion in DMD/BMD patients from North India as compared to other populations particularly in Asia. Also, more than 80% of the mutations are clustered at the central hot spot region between exon 43 and 52 (ref. 5). This pattern holds true for all caste groups of UP despite the higher incidence of mutations responsible for causing the disease in *brahmins* and *vaishyas*. Exons(s) of the dystrophin gene involved in the patients from various caste groups are also not appreciably different (Figure 3). Moreover, no specific exon(s) deletion in the dystrophin gene could be correlated with particular caste groups analysed[14].

Molecular basis for two phenotypes resulting from mutation in the same gene has been explained by reading frame hypothesis. According to it, deletional mutations changing the translational reading frame of dystrophin should cause severe form of the disease (DMD) but deletions still maintaining the frame result in milder form (BMD)[15]. We have determined the in-frame and frame-shift deletions in various caste groups. In *brahmins*, in-frame deletions account for about 42% of their deletional cases while in others, it ranges from 14% to 23% (Table 1). However, in spite of in-frame deletions, many *brahmin* patients have severe form of the disease (DMD).

Table 1. Incidence of DMD/BMD in different caste groups of Uttar Pradesh

Caste	Population* per cent in UP	No. of affected families (patients)	Gene deletion observed in families	Gene deletion in-frame/out-frame
Brahmin	11	46 (60)	33	14/19
Kshatriya	09	19 (20)	10	2/8
Vaishya	05	21 (28)	13	3/10
BC/SC	50	51 (60)	26	5/21
Muslims	16	17 (18)	07	1/6
Others	09	02 (03)	00	0/0

*% Population of different castes was obtained from *India Today*, 15 May 1996.

Various factors have been attributed for population-based variations in the dystrophin gene mutations. Presence of repetitive elements like d*A*–d*T* stretches and transposon-like sequences have been implicated in the high frequency of deletional mutations at the central hot spot region of the dystrophin gene[16,17]. It is possible that due to phenomena like genetic drift, local DNA environment might be different in various caste groups. Such differences can probably account for variations in occurrence of pathogenic mutations at the DMD locus. It would be of interest to further explore specific haplotypes for Xp^{21} locus (location of dystrophin gene) in different caste groups. These observations may have implications for genetic counselling programmes in our country.

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Studies on antianaphylactic activity of fractions of *Albizzia lebbeck*

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Two fractions (F079 and F080) of the hot aqueous extract of stem bark of *Albizzia lebbeck* have been evaluated for antianaphylactic/antiallergic activity *in vitro* and *in vivo*. Both fractions inhibited antigen-induced contraction of the sensitized guinea pig ileum (Schultz–Dale phenomenon). The bronchoconstriction induced by the antigen egg albumin in presensitized guinea pig was also inhibited by these fractions in a dose-dependent manner. The fractions, however, did not possess any bronchodilatory effect *per se* in nonsensitized animals.

THE treatment of bronchial asthma is far from satisfactory. A potent drug against bronchial asthma is still wanting. However, there are claims in the traditional systems of medicine for the treatment of bronchial asthma. *Albizzia lebbeck* is a major constituent of the traditional medicines used against bronchial asthma[1]. The decoction of the bark of *A. lebbeck* was found to protect guinea pigs against antigen-induced challenge and there was a marked inhibition of Schultz–Dale phenomenon[2]. Chronic treatment with the bark decoction also protected the sensitized guinea pigs against antigen challenge[3]. It has been observed that the aqueous extract of the plant possesses antiallergic activity. In a previous study we have observed that the hot aqueous extract of the plant showed promising antiallergic activity in PCA (passive cutaneous anaphylaxis) and mast cell stabilizing activity[4]. The most active fraction from this extract was further chromatographed and fractions F079 and F080 showed maximum activity. The antiasthmatic/

antiallergic activities of these fractions cannot be wholly due to smooth muscle relaxant, antihistaminic or antispasmodic activity[2]. To validate the traditional use of the plant, the present study deals with the effect of these fractions on Schultz–Dale phenomenon, egg albumin-induced bronchoconstriction and bronchodilatory activity *per se* (Konzett and Rossler experiment).

The present study was conducted with the aqueous decoction of the stem bark of *A. lebbeck.* Coarsely powdered bark of the tree (1 kg) was heated with water (2 l) over a boiling water bath for 4 h. The aqueous extract was collected by decantation. The process was repeated four times. The combined extract was evaporated to dryness under vacuum at 70°C, leaving a residue (150 g). The dried extract was not fully soluble in water and did not show any clean spots on thin layer chromatogram. A portion of the dried extract (5 g) was macerated with water (4 × 10 ml) and the insoluble residue (1.5 g) was filtered off. The soluble portion was concentrated and charged on a column of XAD – 2 resin (20 g). Elution was carried out successively with water, 20% aq. methanol, 40% aq. methanol, 60% aq. methanol, 80% aq. methanol and methanol, 100 ml each. Eluates of water, 20% aq. methanol, and 40% aq. methanol were combined together to give a water-soluble fraction F079, whereas the eluates of 60% aq. methanol, 80% aq. methanol and methanol were combined together to give a methanol-soluble fraction F080.

Male guinea pigs (350–450 g) were sensitized with egg albumin (EA 10 mg/kg i.p.) on day 1 and 3. On the day 14, animals were sacrificed and ileal strips from each animal were mounted in isolated organ bath using Tyrode's solution at 37 ± 0.5°C. Tissue sensitivity was tested with histamine hydrochloride (0.05 μg/ml). Antigen was added to the 30 ml bath (EA 3 μg/ml) and responses recorded for 90 s. Various concentrations of *A. lebbeck* fractions (F079/F080) were added to see the inhibition or potentiation of antigen-induced concentration of ileal strips. IC_{50} (inhibitory concentration) were calculated.

Male guinea pigs (350–450 g) were sensitized with antigen on day 1, 3 and 5 (EA, 100 mg i.m.). After 4 weeks, the animals were transferred to the aerosol chamber and a fine mist of the antigen (EA 5%) was sprayed into the chamber under a constant pressure (280 mmHg) till the preconvulsive breathing started. The time was noted down. *A. lebbeck* (F079/F080) fractions were administered orally (50 mg/kg) and disodium cromoglycate (50 mg/kg i.p.) 1 h prior to challenge and the time for showing preconvulsive breathing was recorded. Per cent protection from anoxic convulsion was

Table 1. Inhibition of antigen-induced contraction (Schultz–Dale phenomenon) by fraction of *A. lebbeck* ($n = 3$). (Dose of egg albumin = 3 μg/ml)

Fraction	Conc. (μg/ml)	Per cent inhibition of contraction (Mean ± SD)
F079	1.5	24.5 ± 5.19
	2.0	41.85 ± 8.17
	2.5	71.9 ± 7.67
F080	1.0	25.16 ± 5.10
	1.5	61.36 ± 3.27
	2.0	79.66 ± 1.52

Table 2. Per cent protection from egg albumin induced anoxic convulsion by *A. lebbeck* ($n = 3$)

Fraction	Dose (mg/kg p.o.)	Per cent protection from anoxic convulsion
F079	50	62.69 ± 1.99*
F080	50	46.86 ± 1.95*
DSCG	50 (i.p.)	68.33 ± 3.21*

*Significant activity ($p < 0.05$).

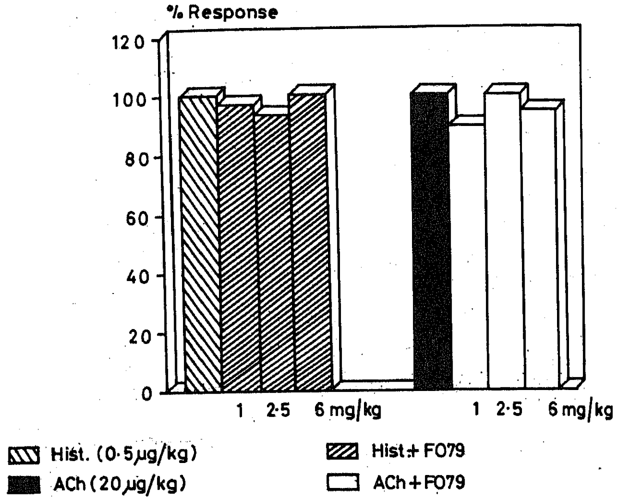

Figure 1. Effect of *A. lebbeck* fraction F079 on bronchoconstriction in guinea pig Konzett–Rossler preparation (Not significant); $n = 3$.

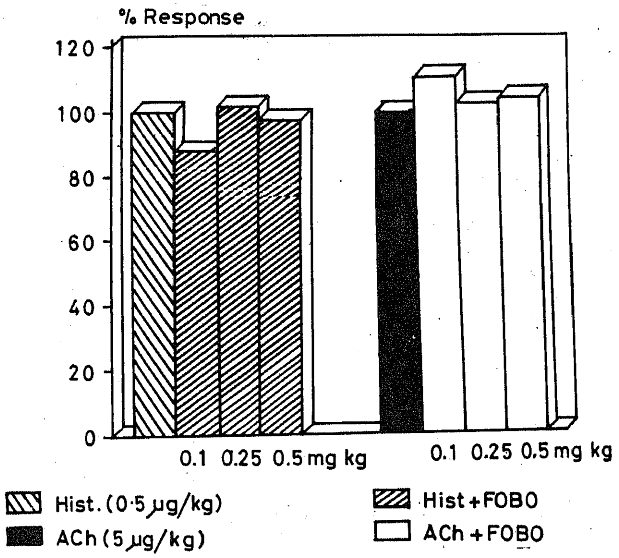

Figure 2. Effect of *A. lebbeck* fraction F080 on bronchoconstriction in guinea pig Konzett–Rossler preparation; $n = 3$.

calculated by the formula $(1 - T_1/T_2 \times 100)$ where T_1 is the mean of preconvulsion time 2 days before treatment and T_2 is the mean of preconvulsion time 2 days after the treatment[5].

In Konzett preparation, normal male guinea pigs (350–450 g) were used. They were anaesthetized with urethane (0.6 ml/100 g, 25% solution) and tied on the operating table. The trachea was cannulated and constant volume of air (6–10 cc) from the respirator was passed through the tracheal cannula[6]. The bronchoconstriction was induced by histamine (0.5 µg/kg) or acetylcholine (20 µg/kg). Thereafter, varying concentrations (0.1 to 5 mg/kg) of *A. lebbeck* (F079/F080) were injected intravenously to see the effect on spasmogen-induced bronchoconstriction.

Statistical analysis was done using Student's *t* test. The effect of *A. lebbeck* on Schultz–Dale phenomenon in guinea pig is summarized in Table 1. It was seen that *A. lebbeck* treatment significantly inhibited antigen-induced contraction in a dose-dependent manner while the response to histamine was maintained. IC_{50} (inhibitory concentration) was also calculated, which was 2.2 and 1.3 µg/ml with F079 and F080 respectively.

When *A. lebbeck*-treated presensitized guinea pigs were exposed to antigen in the aerosol chamber, there was a significant protection from anoxic convulsion. The results are summarized in Table 2. The fraction F079 showed 62 per cent protection which is comparable to the standard drug disodium cromoglycate, whereas with the fraction F080, it was not very significant.

In the Konzett preparation, *A. lebbeck* fractions (F079/F080) did not produce any inhibition or potentiation of histamine-induced bronchospasm. Fraction F079 (0.5, 2.5 and 5 mg/kg) and fraction F080 (0.1, 0.2 and 0.5 mg/kg) did not antagonize histamine and acetylcholine-induced bronchospasm (Figures 1 and 2).

The beneficial effect of *A. lebbeck* bark in bronchial asthma is not due to any significant bronchial smooth muscle activity[3]. In the studies from our laboratory on the mast cells of albino rats, it was found that it has cromoglycate-like action, inhibiting degranulation of sensitized mast cells when exposed to the antigen. In studies on the Schultz–Dale reaction in guinea pigs, there was dose-dependent inhibition of antigen-induced contraction of sensitized ileum treated with *A. lebbeck*. This effect could be due to stabilization of guinea pig intestinal mast cell membrane, inhibiting antigen-induced histamine release or due to inhibition of phenomenon of sensitization[7]. In the peritoneal mast cells of sensitized albino rats treated with *A. lebbeck*, there is inhibition of degranulation when exposed to antigen *in vitro*[2]. Thus *A. lebbeck* has the mast cell-stabilizing activity. This may be responsible for inhibition of the release of mediators in Schultz–Dale reaction in guinea pigs also.

Similarly, when the sensitized guinea pigs were exposed to the antigen spray in the aerosol chamber, there was either prevention of anaphylaxis or prolongation of the latent period of preconvulsive breathing. The protection from preconvulsion is comparable to the standard drug disodium cromoglycate. In previous studies when normal animals were exposed to the histamine aerosol[2], neither the fractions nor the standard drug could protect the animals from anoxic convulsion. These results indicate that *A. lebbeck* protects the sensitized guinea pigs from anoxic convulsions in a manner similar to disodium cromoglycate.

In the Konzett Rossler preparation, the plant fraction does not protect the normal guinea pigs from histamine and acetylcholine-induced bronchospasm even in high doses.

Thus, the present study shows that *A. lebbeck* fractions can protect the sensitized guinea pigs against antigen-induced bronchospasm, inhibit Schultz–Dale phenomenon of guinea pigs, but are devoid of any bronchodilatory activity. The fractions, therefore, contain the active constituents responsible for antiasthmatic activity of the plant. Further study is in progress to isolate and characterize the active principle(s) and to establish their mode of action.

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Effect of supplementary ultraviolet-B radiation on young wheat seedlings

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Effect of supplementary UV-B radiation (1 mW m^{-2}) on photosynthesis, flavonoid content and anatomical changes was studied in young wheat leaves. It was observed that supplementary UV-B radiation did not affect PS II activity, assayed as water to phenylenediamine. However, PS I activity, assayed as reduced dichlorophenol indophenol to methyl viologen, showed an increase. UV-B treatment resulted in qualitative and quantitative changes in UV-B absorbing phenolic compounds. Flavonol (Kaempferol) and caumarin showed quantitative increase due to supplementary UV-B exposure to the wheat leaves. Synthesis of cinnamic acid was observed only after 4 days of UV-B treatment. UV-B treatment resulted in significant anatomical changes in the leaves. There was a massive increase in the cutin synthesis. Epidermal cells were largely destroyed, while hypodermal cells were seen to replace the epidermal cells.

HIGH fluence rate of short wavelength (<320 nm) reaching the earth is increasing due to ongoing depletion of stratospheric ozone layer[1]. Plants use sunlight for photosynthesis and as a consequence are exposed to the ultraviolet radiation. The enhanced levels of UV-B radiation induce damage to various plant processes, particularly resulting in lowered photosynthesis and inhibited growth[2]. A large number of plants exhibit UV-B response, but the photoreceptor responsible for important biochemical parameters such as photosynthesis remains to be conclusively identified[3–6]. The damages due to UV-B radiation can be classified into two categories: damage to DNA (it can cause heritable mutations) and damage to physiological processes such as photosynthesis and proteins, subsequently resulting in decline in plant productivity[7].

To protect itself against enhanced UV radiation, plants have developed protective responses which may prevent damage to the various physiological processes. UV-B-absorbing phenolic compounds (flavonoids) are thought to protect photosynthetic tissue by acting as screening pigments[8,9]. Besides, anatomical changes may occur to reduce the level of UV-B reaching the susceptible site(s).

In this study, we report the effect of UV-B radiation on photosynthesis (PS II activity), qualitative and quantitative changes in UV-B absorbing compounds (flavonoids and other phenolic compounds) and anatomical changes that were observed in wheat leaves in response to UV-B treatment.

Wheat (*Triticum aestivum* L. cv. HD 2380) seeds from Indian Agricultural Research Institute, New Delhi, were grown in 10 cm × 10 cm plastic pots containing vermiculite, and bottom-irrigated routinely using 1/2 strength Hoagland's solution. Plants were grown in a growth chamber illuminated with incandescent bulbs and fluorescent tubes for 12 h photoperiod having a photon flux density (PFD) of 200 μmol m^{-2}s^{-1}, measured with a radiometer (model LI-189, Licor, USA). The day/night temperature was maintained at 25 ± 2°C.

Pots containing 4-day-old wheat seedlings were transferred to another growth chamber with identical PFD, photoperiod and temperature described under growth conditions and were supplemented with UV-B radiation. The source of UV-B was a Vilbour-Lourmat (France) T-6 M source (1 × 6 W fluorescent tube with a permanent filter) with a peak at 312 nm (Figure 1). The UV-B radiation was measured using a UV-B radiometer (with a sensor for 312 nm) from the same manufacturer (Vilbour-Lourmat). The intensity of UV-B irradiation was measured at base, middle and top of the seedlings by placing the sensor of the UV-B radiometer in close contact with the leaves in the middle of the pot. The average intensity of UV-B radiation was 1 mW m^{-2}. Care was taken to maintain the average level of UV-B irradiance at 1 mW m^{-2} during the treatment while plants were growing by changing the distance between source and seedlings and placing the bandage cloth in between.

Isolation of chloroplast from control and treated wheat seedlings and PS II activity (assayed as H_2O to phenylenediamine; O_2 evolution) and PS I activity (assayed as $DCIPH_2$ to MV; O_2 uptake) were assayed according to Sharma and Singhal[10].

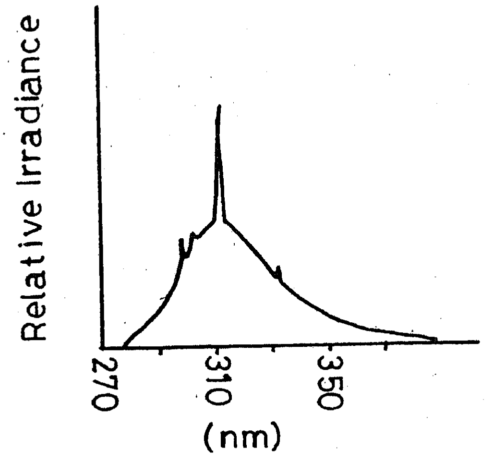

Figure 1. Spectral curve of UV-B source. Filter life time unlimited. Source intensity 6 mW cm^{-2}.

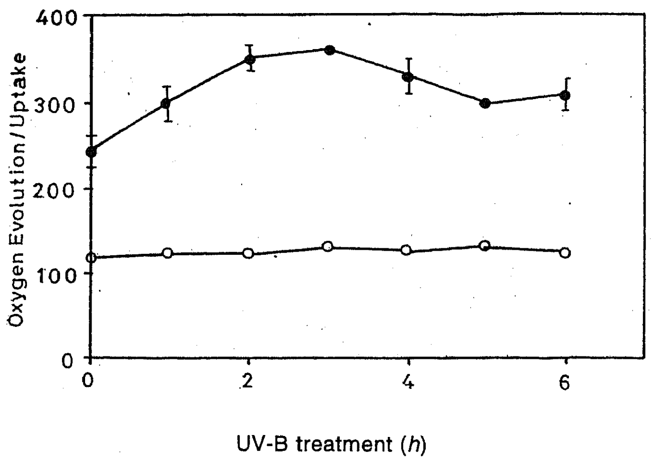

Figure 2. Effect of UV-B radiation (1 mW m^{-2}) on PS II (–O–) assayed as H_2O to phenylenediamine (oxygen evolution) and PS I activity (–●–) assayed as reduced dichlorophenol indophenol to methyl viologen (oxygen uptake). The PPFD during assays was 1200 μmol m^{-2} s^{-1}. Rates are expressed as μmol O_2 evolved or consumed/mg chlorophyll/hour. Each point represents a mean value of 4 experiments. Standard error (±8) for PS II is not visible on the scale.

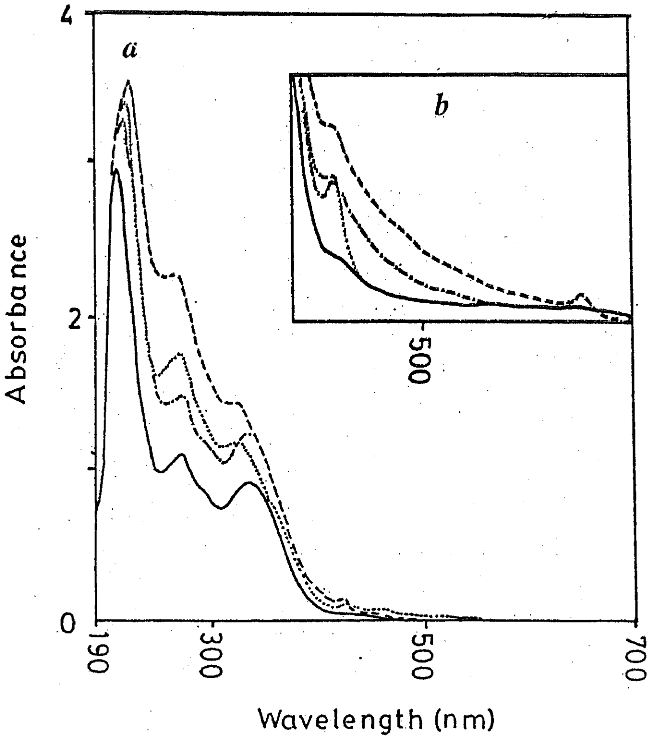

Figure 3. Effect of supplementary UV-B radiation on quantitative change in flavonoid contents in wheat seedlings. *a*, Spectral scan at 1:5 dilution; *b*, Spectral scan at 1:3 dilution. Control, i.e. 0 day UV-B treatment (------), 3 day UV-B treatment (.........), 5 day UV-B treatment (–.–.–.–.–.–) and 7 day UV-B treatment (————).

Fresh leaves (2 g) were excised and homogenized in 20 ml of 80% methanol with 1% HCl (final volume) in a mortar. The homogenate was incubated for 2 h at room temperature in the dark and centrifuged at 1600 *g* for 20 min. The supernatant was collected for absorption spectra, paper chromatography and HPLC (Spectraphysics).

Spectral scan of UV-B absorbing compound was carried out with diluted extract using spectrophotometer (Shimadzu UV240).

For paper and HPLC analysis, 5 ml of each sample was fully dried by flushing N_2 and re-dissolved in 200 μl of methanol. 20 μl of sample was loaded on a Whatman filter paper no. 1 and resolved using *n*-butanol:acetic acid:water (6:1:2) at room temperature and observed under UV photoilluminator.

Identification and separation of flavonoids was carried out using HPLC (Spectraphysics) with a C18 reverse-phase column (ET 250/4 Nucleosil 100-5C_{18} ODS) Spectraphysics SP 8800 ternary HPLC pump with SP 4270 integrator and Spectra 100 variable wavelength detector. The 100 μl of re-dissolved sample was diluted in 1 ml methanol and 25 μl of this was injected into the HPLC column. The separation was carried out using an isocratic system (0.5% phosphoric acid and methanol in 1:1 ratio) over 20 min with a 1.0 ml/min flow rate at 280 nm and 418 nm at room temperature.

Wheat leaves were fixed in a solution containing 50% alcohol, 10% acetic acid and 10% formic acid. The fixed material was passed through a series of 90, 70, 50, 30 and 100% of alcohol for 2 h to overnight duration. This was followed by a series (50, 70, 85, 95 and 100%) of tertiary butyl alcohol (TBA) for 2 h duration followed by TBA and paraffin oil (1:1) for 2 h. The plant material was then fixed in paraffin wax (60°C) blocks and cut into sections using microtome (Sipcon SP1110). Sections were stained with safranine and fast green and mounted in the DPX. The anatomical observations were recorded by light microscope (Leica) with photographic attachment (Leica).

It was observed that plants treated with supplementary UV-B radiation did not result in significant changes in the PS II activity, assayed as H_2O to phenylenediamine (PD), compared to the plants grown without supplementary UV-B radiation (0 h). However, PS I activity assayed as dichlorophenol indophenol ($DCIPH_2$) to methyl viologen (MV), showed a significant increase in the activity in UV-B supplemented plants compared to non-supplemented plants (Figure 2).

Absorption spectra of 80% methanolic extract with 1% HCl is shown in Figure 3 *a*. It was observed that plants treated with UV-B radiation showed an increase in the absorption peaks at 210–220 (caumarin). UV-B treated plants showed a slight shift in the absorption peak of caumarin from 210 to 220 nm, 270–280 nm (Kaempferol) and 320–330 nm (flavone). Absorption spectra of concentrated extract also showed slight changes in the absorption at 418 and 660 nm (anthocyanin) in the visual region (Figure 3 *b*, Table 1).

UV-B-absorbing compounds showed qualitative changes in response to UV-B radiation which were

Table 1. Effect of supplementary UV-B radiation (1 Mw m^{-2}) in combination with visual radiation (60 μmol m^{-2} s^{-1}) at 25°C on flavonoids and its derivatives in wheat seedlings. Comparative data of spectrophotometric analysis and paper chromatogram seen under visual and UV-B transilluminator before and after NH_3 fuming. According to Swain[11] the colour of spot under visible and UV light with and without NH_3 treatment of the chromatogram indicate type of flavonoid

Spectrophotometric data		Paper chromatogram					
			Spot colour				
					+NH_3 fumes		
Lamda max	Compound	*Rf* × 100	Visible	UV	Visible	UV	Compound
270	Flavonol (Kaempferol)	84	Brownish yellow	Brownish yellow	Brownish yellow	Brownish yellow	Flavonol
210	Caumarin	79	Colourless	Blue	Colourless	Blue	Caumarin
		59	Colourless	Lightblue arrowshape	Colourless	Light blue arrowshape	Cinnamic acid
418	Anthocyanin	56	Brownish yellow	Brownish yellow	Brownish yellow	Brownish yellow	Anthocyanin
320–330	Flavone	34	Colourless	Purplish brown	Yellow	Yellow	Flavone
655–660	Anthocyanin	31	Brown	Purplish brown	Bright yellow	Bright yellow	Anthocyanin

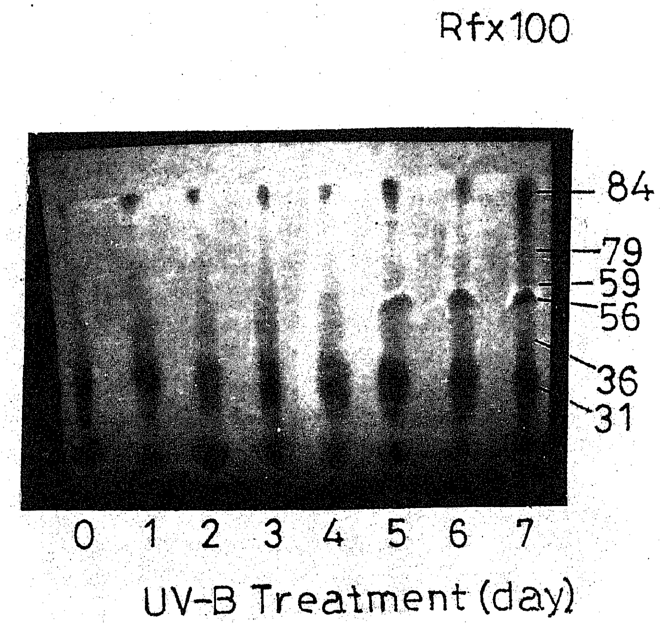

Figure 4. Effect of supplementary UV-B radiation for 0 to 7 day (left to right in the figure) on flavonoid content in wheat seedlings. The *Rf* value and colour composition under visual light with and without NH_3 fumes and nature of compounds are given in Table 1.

determined by paper chromatography. All major compounds visualized in extract from UV-B-irradiated plants were seen in control except cinnamic acid which appeared after day 4 of UV-B treatment (Figure 4). The possible group of flavonoids were determined on the basis of *Rf* value, and colour characteristics under visual and UV transilluminator (with and without treatment with NH_3 fumes) and their absorption spectra according to Swain[11] (Table 1).

HPLC analaysis of methenolic extract at 280 nm showed qualitative changes in the flavonoids. UV-B exposure resulted in an appearance of two new peaks (peak no. 3 and 7; cinnamic acid and anthocyanin respectively) compared to the control. Peaks 1 and 2 (kaempferol and caumarin respectively) showed increase due to the supplementary UV-B exposure. HPLC analysis using visual range (418 nm) also showed significant changes in the anthocyanin (Figure 5 *b*). The nature of new peaks observed in Figure 5 *b* could not be determined.

The leaf section showed a massive increase in the deposition of cuticle wax in the UV-B treated leaves compared to control (Figure 6 *a–d*). Epidermis was damaged to a greater extent (Figure 6 *b* and *c*). Mesophyll cells were converted into hypodermal cells (Figure 6 *c*) to replace the damaged epidermal cells (Figure 6 *c* and *d*).

Our study shows that supplementary UV-B radiation did not cause significant changes in the PS II activity but the PS I activity showed a significant increase. Qualitative and quantitative changes in the flavonoids and other phenolic (phenyl propane) derivatives using analytical techniques such as spectrophotometry and chromatography were also observed.

Response of photosynthetic apparatus to UV-B radiation has been studied by several workers[9,12–16]. A few reports have suggested photosystem II as the UV-B-sensitive component[14,15], but the action spectrum of UV-B does not suggest a specific target molecule[5,6,13].

Jie He *et al.*[15] observed decrease in PS II activity measured as *Fv*/*Fm* ratio in rice and pea leaves. However, they have not measured photosynthetic electron

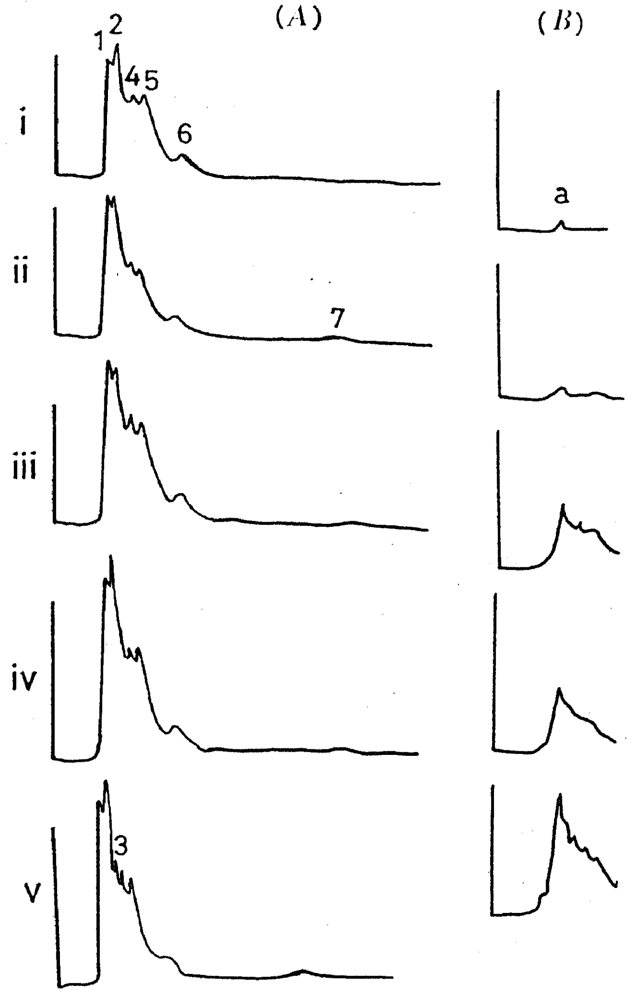

Figure 5. HPLC analysis showing qualitative and quantitative changes in wheat seedlings under supplementary UV-B exposure for 0 (i), 2 (ii) 3, (iii) 5 (iv) and 7 (v) days. ***A***, HPLC analysis at 280 nm. 1, Kaempferol; 2, Caumarin; 3, Cinnamic acid; 4 and 5, Flavone; 6 and 7, Anthocyanin; ***B***, HPLC analysis at 418 nm. *a*, anthocyanin. Other peaks seen are not identified.

transport. Middleton and Teramura[9] showed that the reduction in photosynthesis, under UV-B radiation, was primarily associated with a stomatal limitation rather than the damage to PS II.

In our study we have not observed any damage to PS II activity (Figure 2). This may be due to low UV-B fluence rate (1 mW) for a short duration (up to 6 h only). Also, the effect of supplementary visual radiation ($200\ \mu\mathrm{mol\ m^{-2}\ s^{-1}}$) during the UV-B exposure, may have resulted in preventing the damage due to PS II. There have been reports that UV-B-mediated effects are dampened by the background intensities of photosynthetic active radiation[17–20].

The increase in the PS I activity may be owing to micro-environmental changes in the thylakoid membrane (lipids/protein) due to oxidative damage[21], making reduced DCIP more assessable to the site of action (electron donation).

In this study we observed quantitative and qualitative changes in the UV-B absorbing flavonoids in response to supplementary UV-B radiation (Figures 3–5). It has been suggested that flavonoids and phenyl derivatives are involved in the protection against UV-B exposure in higher plants[8,12,15,22,23]. Flavonoids and anthocyanins absorb UV-B radiation and these generally accumulate in the epidermis. Here they could keep UV-B radiation from reaching photosynthetic tissues and other sensitive sites (protein, DNA, etc.).

Lois[8] observed that irradiance of *Arabidopsis* with UV-B light resulted in accumulation of flavonoids in the aerial parts of the plants. He correlated this increase with the protection (less damage to plant growth) against UV-B damage.

Lois and Buchanan, working with *Arabidopsis* mutant deficient in flavonoids accumulation, found that mutant displayed a dramatic increase in the sensitivity (reduced plant growth) to UV-B radiation compared with wild type, suggesting a protection role for flavonoids against UV-B radiation.

Li *et al.*[16], working with *Arabidopsis* mutant with defects in the synthesis of flavonoids, found them more sensitive (less dry weight) to UV-B than the wild type when grown under UV-B radiation.

Jie He *et al.*[15], working with rice and pea plants, found that rice cultivar showed less decrease in *Fv/Fm* ratio as compared to pea. They correlated this to the higher levels of UV-B absorbing compounds in rice as compared to pea. This indicates a role of flavonoids in the protection against UV-B in higher plants.

In this study we also observed a slight shift in the absorption peak of caumarin (210–220 nm; Figure 3 *a*). This shift in lambda maximum for caumarin may be due to its glycosylation[23].

Our study shows massive increase in the cuticle layer in the leaves in response to UV-B radiation (Figure 6 *a*–*d*). Earlier studies have shown that UV-B radiation causes epidermal deformation[12], changes in cuticular wax composition[24]. These changes may result in preventing harmful UV-B radiation to reach sensitive site. Hahlbroack and Scheel[25] and Robberecht and Caldwell[26] reported that cutin deposition along with flavonoid synthesis in upper epidermis could block transmittance of 95–99% of incoming UV-B radiation.

The increase in the cuticle layer under UV-B exposure may prevent the penetration of the UV-B radiation by reflecting large amounts of it. Development of hypodermal cells from mesophyll cells (Figure 6 *c* and *d*) may replace the damaged epidermis. Also the development of hypodermal cell may prevent the UV-B radiation from reaching the more sensitive sites by providing layer(s) of cells which accumulate large amounts of

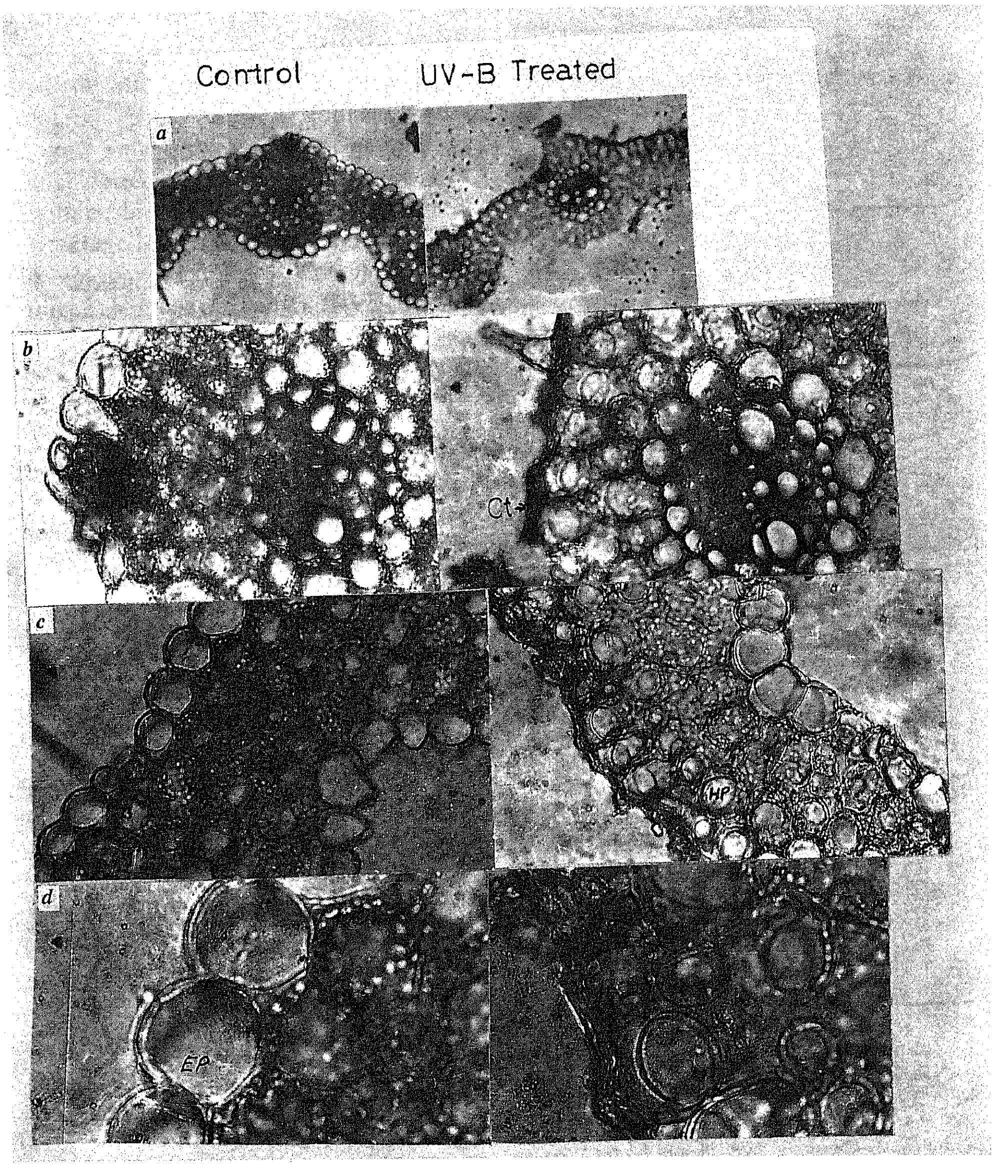

Figure 6. Effect of supplementary UV-B radiation on leaf anatomy. ***a***, TS of mid rib portion of control and UV-B treated leaf respectively (10×); ***b***, TS of mid rib portion of control and UV-B treated leaf respectively (40×). Ct, cuticle; ***c***, TS of control and UV-B treated leaf (non-midrib region) respectively (40×). HP, hypodermal cell; ***d***, TS of control and UV-B treated leaf respectively (100× oil). EP, Epidermal cell.

phenolic compounds (flavonoids). This may further help in absorbing the UV-B radiation, thus protecting more sensitive sites, such as DNA and enzymes, from UV-B radiation.

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Temporal patterns of visitation among avian frugivores at fruiting strangler figs in a tropical evergreen forest in the Western Ghats, Southern India

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Frugivory observations were conducted at fruiting strangler figs in Karian Shola National Park in the Anaimalai hills from January to March 1993. A total of 123 hours of fruit use by frugivores was observed at fruiting trees of four species of *Ficus*. Frugivore activity was seen throughout the day with a major peak in visitation between 7.00 AM and 9.30 AM and a minor one at 2.30 PM. The common frugivore species exhibited preferences for different species of fruiting strangler figs, occurring in larger numbers at these trees. Of the seven common species of avian frugivores, only two pairs exhibited similar temporal patterns of visitation to the fruiting strangler figs, the Small Green Barbet–Crimsonthroated Barbet pair at *F. drupacea* and bulbul spp.–Golden Oriole pair at *F. microcarpa*. The pressures of competition, past or contemporary may be the reason behind the difference in the temporal pattern of visitation. However, it is unlikely that active competition is the cause since very few inter-specific aggressive interactions were noted. What is unclear is the similar temporal pattern of visitation in the case of the bulbul spp.–Golden Oriole pair at *F. microcarpa*, their preferred tree and the barbet pair at *F. drupacea*, not their preferred tree.

FRUITING fig trees, especially those that are bird-dispersed, attract avian frugivores in large numbers[1]. This has led to a number of studies on frugivore composition and their interactions at this superabundant fruit resource[2–6]. Visitations to the fruiting trees may depend on physiological needs of the frugivores and the local factors peculiar to each fruiting tree. Time spent by a frugivore at a fruiting tree would depend on its nutritional needs, diet and activity budgets. The presence of predators and the interactions among the frugivores could also affect visitation rates[7,8]. Kantak[7] surmised that the different visitation patterns of the common frugivores could be due to interference competition. This paper presents the results from a study of temporal partitioning of the fruit resource among avian frugivores visiting fruiting strangler figs in a tropical evergreen forest in the southern Western Ghats, India. The null hypothesis of no differences in the temporal patterns of visitation was tested against the alternative hypothesis of temporal partitioning of the fruit resource by the common frugivores.

The study was conducted from 21 January to 31 March 1993, at Karian Shola National Park in the Indira Gandhi Wildlife Sanctuary, Pollachi district, Tamil Nadu, India. The Karian Shola National Park (10°27'N, 76°51'E; altitude c. 765 m) is spread over 506 ha of tropical evergreen forest. It is contiguous with similar forest across the Kerala–Tamil Nadu border in the Parambikulam Wildlife Sanctuary. It is classified as the west coast tropical evergreen forest with the characteristic tree species being *Hopea parviflora* and *Messua ferrea*[9]. The national park is surrounded by moist deciduous forests and teak plantations. Most of the precipitation in this region occurs during the south-west monsoon (from June to August) but the effect of the north-east monsoon (during November and December) is also felt. This area received 1778.2 mm of rain in 1992 and 27.8 mm during the three-month study period.

A total of 123 hours of frugivory observations was conducted at three trees of *Ficus drupacea* (67 h), one tree of *F. microcarpa* (40 h) and two trees of *F. amplissima* (16 h). Observations were conducted between 7.00 AM and 5.00 PM. The bird species and their numbers were noted during every alternate 5-minute period.

The trees chosen for observations had to satisfy certain criteria. They had to have large synchronously ripening fruit crops; usually bird-dispersed fruiting characteristics in the old world tropics[1]. The trees had to be relatively shorter and located in more open areas. In

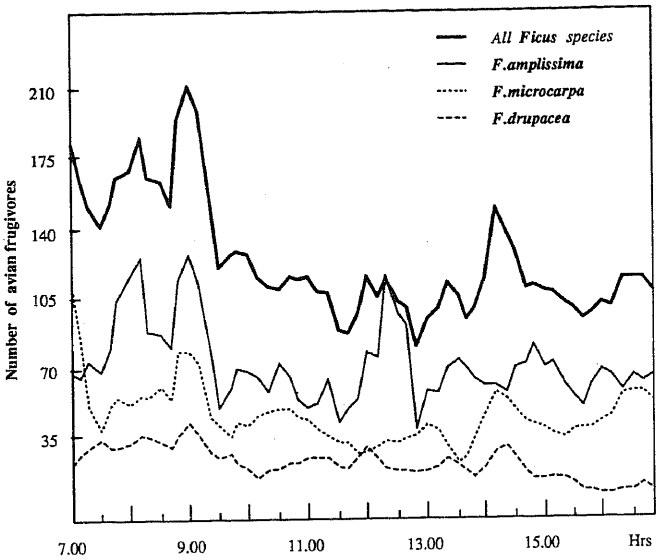

Figure 1. Temporal pattern of visitation of avian frugivores at fruiting *Ficus* trees through the day. The data presented here represent a four-day aggregate of the number of avian frugivores visiting the three *Ficus* species and a 13-day aggregate for all the *Ficus* species combined.

addition, the foliage density had to be low. This was essential to facilitate the identification of important frugivores like the Greyfronted Green Pigeons (*Treron pompadora*) and barbets (*Megalaima* spp.) which are small and green in colour and to obtain a reasonably accurate count of birds in general. This strongly constrained the number of trees that could be observed. Most of the sampled trees were present on the edges of clearings and in riparian areas within the evergreen forest.

Although these trees were chosen based on the above criteria, it is unlikely that this selection would have in any way affected the visitation of the birds both in terms of species as well as numbers to the trees. This is corroborated by the fact that despite the difference in the number of hours of observation at each species of *Ficus*, the number of frugivore species recorded was around the same (19 species were recorded at *F. amplissima*, 24 *F. drupacea* and 26 at *F. microcarpa*). Also, the seven common species that have been used for analysis of frugivore visitation pattern were recorded in fair numbers at all three *Ficus* species[10].

The visitation patterns of all the avian frugivores were studied through the day. For analysis of differences in temporal patterns of visitation, seven of the most common frugivores namely Malabar Grey Hornbill (*Tockus griseus*), Greyfronted Green Pigeon (*Treron pompadora*), Small Green Barbet (*Megalaima viridis*), Crimsonthroated Barbet (*M. rubricapilla*), the bulbuls (*Pycnonotus melanicterus gularis, P. jocosus* and *Hypsipetes indicus*), Golden Oriole (*Oriolus oriolus*) and Fairy Bluebird (*Irena puella*) were considered. The three species of Pycnonotidae were grouped together to improve the statistics as the visitation frequencies of the individual species were low. If the patterns of visitation of the three species were different, their combined data would have yielded smoother pattern. However, the combined data show a definite periodic pattern in their visitation which justifies the assumption that the visitation patterns of the three species are similar.

The number of days during which observations were conducted was not the same for all the time intervals between 7.00 AM and 5.00 PM. The bird numbers in each interval have been scaled to the median value of 13 days to analyse the temporal patterns across a day. The analysis was repeated for each of the three strangler fig species. The bird numbers were smoothened by taking an average over three successive time intervals.

A multinomial χ^2 goodness of fit test was employed to check the null hypothesis of no differences in the patterns of visitation of the common frugivores, taken a pair at a time. In order to improve the reliability of the χ^2 statistic, two constraints were imposed during analysis. Only those time intervals in which the expected number of birds was greater than 2.0 were considered. This severely reduced the number of usable time intervals, especially when the data from different *Ficus* species were considered separately. This in turn resulted in inordinately high values of significance for the null hypothesis which was in fact an artifact due to bad statistics. To avoid this artifact, only the results of those bird pairs which had at least 30 usable time intervals in common (out of a maximum of 60 through the day) have been considered[11].

The fruiting strangler figs had a continuous stream of frugivores right through the day with a major peak in frugivore activity between 7.00 AM and 9.30 AM and a much smaller one at around 2.30 PM (Figure 1). The pronounced early morning peak in frugivore activity may have been due to the increased food requirements of the frugivores at the start of a day. It seems likely then, that there will be another peak in frugivore activity at the fruiting strangler figs at the end of the day, before the frugivores turn in for the night.

The avian frugivores preferred different species of *Ficus*, occurring in large numbers at these species (Table 1). The Malabar Grey Hornbill preferred *F. drupacea*, the Green Pigeons and the barbets preferred *F. amplissima* while the bulbuls, Golden Orioles and Fairy Bluebirds showed a preference for *F. microcarpa*. It is well known that bird-dispersed figs when ripe are harvested by various size of birds, since even those with smaller gape sizes can eat the soft fruit by pecking out small pieces[12]. However, in terms of optimal utilization of the food resource, it is likely that a frugivore will try to eat the largest possible fig convenient given its gape size rather than ingest smaller figs or take bites of larger figs. We see such a trend here with the Malabar Grey Hornbill, the largest of the frugivores preferring the large fruits of *F. drupacea* (1.5–2.0 cm across[13]) while

Table 1. Mean number of avian frugivores at fruiting *Ficus* trees

	Mean number of birds/10 min interval								Mean of days observed
	MGH	GP	SGB	CTB	BUL	FBB	GO	TOT	
Total	0.80	1.79	1.01	2.40	0.78	0.32	0.89	9.47	13
F. amplissima	0.06	3.88	2.47	10.17	0.30	0.00	0.09	18.05	2
F. drupacea	1.11	0.77	0.64	1.48	0.18	0.15	0.04	5.45	7
F. microcarpa	0.38	2.65	0.85	0.61	1.74	0.58	2.50	11.14	4

MGH, Malabar Grey Hornbill; GP, Greyfronted Green Pigeon; SGB, Small Green Barbet; CTB, Crimsonthroated Barbet; BUL, Bulbul spp.; FBB, Fairy Bluebird; GO, Golden Oriole; TOT, Sum of all the avian frugivores.

Table 2. The p-values from the χ^2 test of the null hypothesis of no temporal variation in visitation of pairs of common avian frugivores

Birds species	MGH	GP	SGB	CTB	BUL	GO	FBB
MGH	(626)			*Combined data from all trees.*			
GP	<0.005	(1401)				*df* = 59	
SGB	<0.005	<0.005	(792)				
CTB	<0.005	<0.005	ns	(1878)			
BUL	<0.005	<0.005	= 0.03	= 0.095	(611)		
GO	<0.005	<0.005	<0.005	= 0.017	ns	(699)	
FBB	insf.data	insf.data	insf.data	insf.data	insf.data	insf.data	(251)

Bird species	GP	SGB	CTB
			F. amplissima
GP	(446)		*df* = 59
SGB	insf.data	(297)	
CTB	<0.005	<0.005	(1221)

Bird species	GP	BUL	GO
			F. microcarpa
GP	(638)		*df* = 59
BUL	<0.005	(418)	
GO	<0.005	ns	(600)

Bird species	MGH	GP	SGB	CTB
MGH	(467)			*F. drupacea*
GP	<0.005 *df* = 47	(324)		
SGB	<0.005 *df* = 53	insf.data	(272)	
CTB	<0.005 *df* = 56	<0.005 *df* = 54	ns *df* = 57	(622)

The numbers within parentheses are the sample sizes of the corresponding birds. MGH, Malabar Grey Hornbill; GP, Greyfronted Green Pigeon; SGB, Small Green Barbet; CTB, Crimsonthroated Barbet; BUL, Bulbul spp.; GO, Golden Oriole; FBB, Fairy Bluebird; ns, not significant (>0.1); *df*, degrees of freedom; insf.data, insufficient data.

the bulbuls preferred *F. microcarpa* which has the smallest fruits among the three *Ficus* species.

Excepting two pairs, all the 21 possible pairings of the seven most common frugivores exhibited differing temporal patterns of visitation at the fruiting strangler figs (Table 2). Kantak[7] recorded a similar result at five species of fruiting trees, including a *Ficus* species. The only two pairs which did show similar temporal patterns in visitation were the Small Green Barbet–Crimsonthroated Barbet pair at *F. drupacea* and the bulbul spp.–Golden Oriole pair at *F. microcarpa*. The reasons for the similarity in temporal visitations shown by these pairs of frugivores are not clear. What is puzzling is that while the barbet pair exhibited similar temporal pattern of visitation at a tree which was not their preferred species, the bulbul spp.–Golden Oriole pair exhibited similar visitation pattern at their preferred tree.

One could argue that at their preferred tree, the higher pressures the barbets face from the members of other congeneric species has led to the temporal partitioning of the resource. Therefore at *F. drupacea*, which is not their preferred tree and where competition from the other barbet species is likely to be lower, the closely related barbets have similar patterns of visitation. However, the bulbul spp.–Golden Orioles pair neither have similar habits nor are they closely related[14] to explain the similarity in their visitation pattern to their preferred tree.

The reasons given for temporal variations are many[7,8,15]. Predation pressures and aggression were certainly not the major reasons at Karian Shola National Park as very few instances of predatory attempts (seven in 123 hours of which none were successful) and interspecific aggression (seven in 56 hours of observation) were seen among the birds[10]. While figs are a superabundant resource, they are almost completely utilized by the frugivores[10] and one would expect competition over the resource. Perhaps past competition has moulded present frugivore behaviour and resource partitioning, and manifested in differing patterns of temporal visitations among frugivores.

In conclusion, the fruiting *Ficus* trees were host to frugivores throughout the day, with a higher activity in the early morning and mid afternoon. The major peak in the early morning could be explained by the increased food requirements of the frugivores who have started their day after a long gap in feeding. I would expect another peak in activity prior to sundown when the birds have to stock up for the night. The common frugivores occurred in larger numbers at certain species of *Ficus* and this preference is likely to be based on the optimal exploitation of a fig given the gape size of the frugivore. Most of the avian frugivores, including the two most common at each fruiting tree had differing temporal patterns of visitation to these super-abundant resources. Predation was rare and aggressive interactions between species very few which makes active interspecific competition an unlikely explanation for the differing patterns. What is difficult to explain is the similar temporal pattern of visitation of the bulbul spp.–Golden Oriole pair, at their preferred fruiting tree. Perhaps the biology and activity patterns of different species condition them to particular temporal patterns of behaviour.

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Vegetational and climatic changes during recent past around Tipra Bank Glacier, Garhwal Himalaya

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Palynological studies from Tipra Bank Glacier, UP Himalaya indicate that the climate was warm moist, similar to today's, prior to 720 yr B.P. This then reverted to comparatively cold dry climate around 620 yr B.P. when the glacier might have descended down. The climate changed to warm moist regime again around 460 yr B.P.

PALYNOLOGICAL studies from glacial sediments above tree limit or alpine region of the Himalaya are limited in number[1–3]. Most of the studies on this aspect are confined to temperate and subtropical sites[4,5].

We have attempted here to understand the changes of vegetation around Tipra Bank Glacier and their relationship to glacial fluctuations using pollen data of both surface and subsurface sediments of near present day snout.

Tipra Bank Glacier (Figure 1), one of the major glaciers in Bhyundar Ganga valley of Alaknanda catchment, joins another major glacier, Rataban and together they form a common snout. Bhyundar Ganga river originates from this snout and flows through the famous Valley of Flowers and ultimately joins Alaknanda river at Govindghat near Pandukeshwar. There are fourteen other glaciers which are of small niche types. The detailed climatic data around the site is not available. A general observation in this regard indicates that the

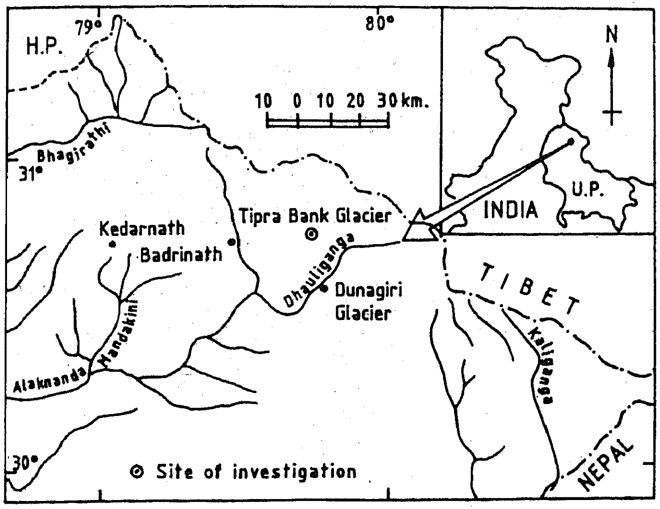

Figure 1. Sketch map showing the site of study.

precipitation is in the form of snow during the major part of the year except for frequent rains during May to September.

The area covers a large number of alpine taxa and these are mostly represented by *Aconitum* sp., *Anaphalis triplinervis*, *Androsace lanuginosa*, *Anemone* sp., *Angelica glauca*, *Artemisia* sp., *Bupleurum himalayense*, *Delphinium brunonianum*, *Geranium wallichianum*, *Geum alatum*, *Gypsophilla cerastoides*, *Heracleum brunonis*, *Meconopsis aculeata*, *Potentilla* sp., *Primula* sp., *Rubus natans*, *Saussurea gossypiphora*, *Saxifraga diversifolia*, other members of Caryophyllaceae, Chenopodiaceae, etc.

The trees in the subalpine zone today extend up to about 2 km south-west of glacier terminus, while scattered scrubs or small trees of *Betula utilis*, *Rhododendron campanulatum*, *Salix flabellaris*, *S. lindienana*, etc. are found even upstream of the glacier snout. Along the timber line, *Abies pindrow* and *Betula utilis* are the dominant species. Besides, the randomly distributed alpine bushes comprise *Acer cappadocium*, *Cotoneaster acuminata*, *Juniperus communis*, *J. squamata*, *Rhododendron lepidotum*, etc.

The samples were collected from the valley floor which consists mainly of glaciogenic deposits overlain at places by talus material. The present-day glacier terminus lies about 3 km NE of the Valley of Flowers. The present-day geomorphological features indicate that this area was occupied by the then Tipra Bank Glacier as evidenced by the existence of lateral, terminal and ground moraines. Towards downstream in Valley of Flowers, these evidences are not visible. Instead, the features indicate a fluvial environment. Quite a few talus cones and fans exist between the Valley of Flowers and the glacier terminus.

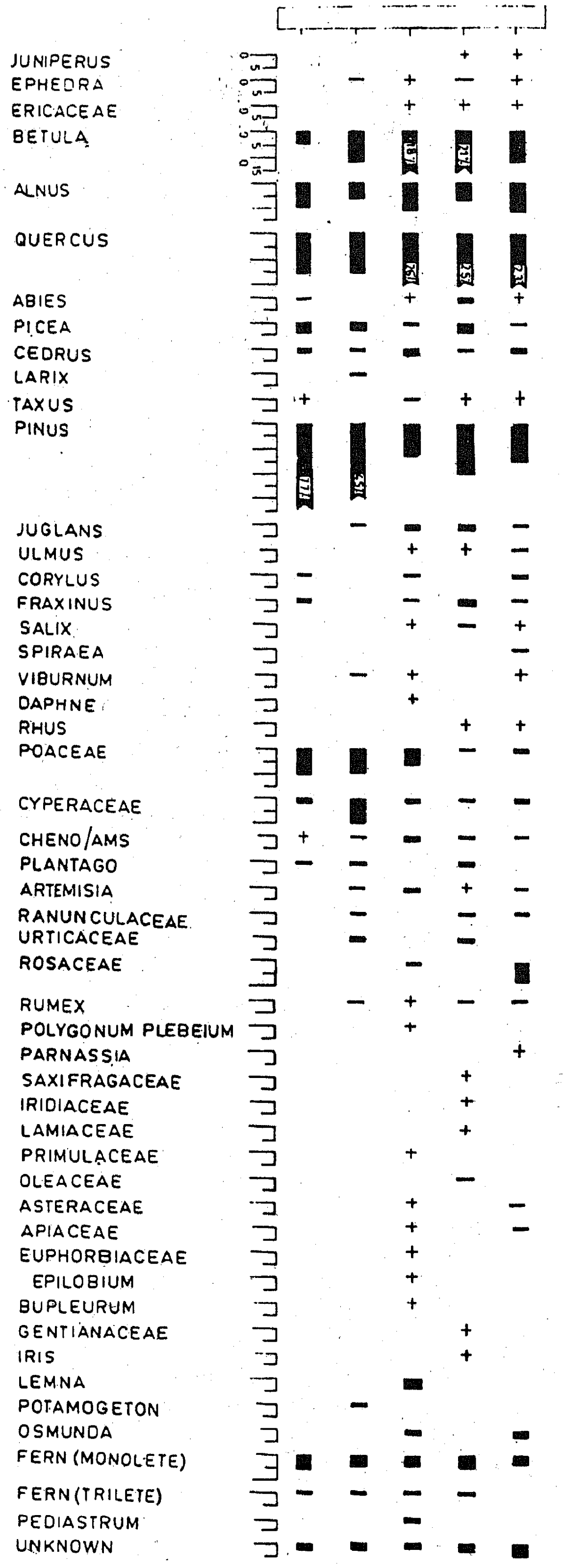

Figure 2. Recent pollen spectra from Tipra Bank Glacier, Garhwal Himalaya.

Table 1. ^{14}C dates and calendar years

Depth	Nature of samples	Dates	(yr B.P.) (^{14}C)	Calendar years (yr AD)
50–60 cm	Black soil	BS-437	460 ± 100	1401–1487 (ca.)
60–70 cm	Black Soil	BS-438	620 ± 90	1275–1408 (ca.)
70–80 cm	Black soil	BS-436	720 ± 90	1235–1300 (ca.)

Materials for the present study have been taken both from surface and a small profile. For surface samples, moss cushions from five places were collected in a transect at different intervals to understand modern pollen vegetation relationship of this site. For the subsurface samples, an 80 cm deep trench was dug along the bank of Tipra stream and 5 samples consisting of black soil at an interval of 5 cm (except a 10 cm interval between 60 and 70 cm depth) were collected for pollen analytical investigation.

Besides, materials were also collected at three different intervals from this trench for radiocarbon dating. These materials have been dated at the Radiometric Laboratory of BSIP. The ^{14}C dates and calendar years are given in Table 1.

The ^{14}C dates are converted to calendar years so that the events of the present study could be related to other evidences under the same time scale. In calibration, 'O' yr BP of ^{14}C date is taken equal to AD 1950. Since the change of atomospheric radioactivity, ^{14}C data need to be recalibrated against tree ring dating. Details of the calibration of ^{14}C date to calendar years have been given elsewhere[6]. Both surface and subsurface samples were analysed using usual pollen analysis technique[7].

Five surface samples (moss cushions) analysed from the vicinity of the snout provide database regarding representation of modern pollen in relation to the extant vegetation (Figure 2). This study shows *Pinus* 15–77% followed by *Quercus* 15–26%, *Alnus* and *Betula* 2–5% each, *Picea* 1–5%, *Juglans* and *Cedrus* 1–4% each and 1% each of *Abies* and *Fraxinus*. The shrubby elements, viz. *Juniperus*, *Rhus*, *Daphne*, and *Spiraea* are extremely low and sporadic. Among the non-arboreals, Poaceae (1–12%), Cyperaceae, Cheno/Ams, *Artemisia*, Ranunculaceae (under 2% each) and *Plantago* and Rosaceae (1–8% each) are comparatively better represented than *Polygonum plebeium*, *Parnasia*, *Iris*, *Epilobium*, Saxifragaceae, Iridaceae, Primulaceae, Oleaceae, Asteraceae, etc. which are represented by under 1% each. Fern spores (monolete 4–6%, trilete 2–3% and *Osmunda* 2%) are also recorded frequently in the samples.

A pollen diagram (Figure 3) has been made only from five pollen spectra which are dealt with below along with their equivalent ^{14}C dates.

Around 720 ± 90 yr B.P. the pollen spectra are characterized by high frequencies of *Pinus* (10–24%)

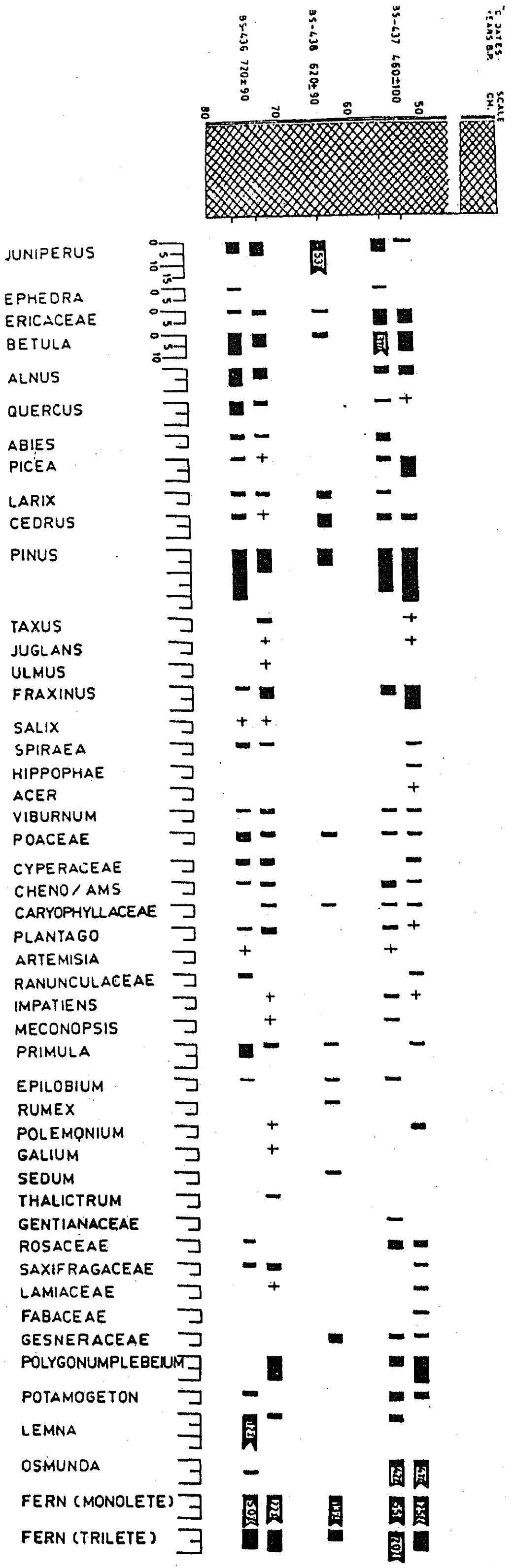

Figure 3. Pollen diagram from Tipra Bank Glacier, Garhwal Himalaya.

followed by *Betula* (5–10%), *Alnus* (4–10%), *Quercus* (2–6%), *Juniperus* (5–7%) and *Fraxinus* (1–5%). The other tree taxa such as *Abies, Cedrus, Larix, Taxus* and Ericaceae (under 2% each) are consistently represented in low frequencies. *Juglans, Ulmus* and *Salix* (under 1% each) are met with scantily. The shrubby vegetation is poorly represented (under 2% each) by *Spiraea* and *Viburnum.*

The non-arboreals are mainly represented by *Primula* (2–7%), Poaceae (2–5%), Cyperaceae, Cheno/Ams, *Plantago* and *Saxifraga* (2–3% each). *Artemisia, Impatiens, Meconopsis, Epilobium, Rumex, Polemonium, Galium, Sedum, Thalictrum,* Rosaceae and Lamiaceae (under 1% each) are extremely low and sporadic. Aquatic vegetation is represented by *Lemna* (2–12%) and *Potamogeton.*

Fern spores (monolete 22–50% and trilete 9–10%) are recorded in preponderance.

During 620 ± 90 yr B.P. in general, a comparatively poor assemblage as well as low frequencies of most of these taxa have been noticed, except *Juniperus* which shows a drastic enhancement (53%). *Cedrus* (6%) and *Larix* (3%) are also slightly better represented, whereas *Pinus* (11%) declines considerably. The ground vegetation is scantily represented by Poaceae (2%) and under 1% each of Caryophyllaceae, *Primula, Epilobium, Rumex* and *Sedum.* Fern spores (monolete 13% and trilete 5%) decline sharply.

During 460 ± 100 yr B.P. *Betula* (10–31%), *Pinus* (20–25%), *Picea* (2–9%) and *Abies* (3%) indicate the increased frequencies, but *Juniperus* (6–1%) declines abruptly. The other taxa such as *Fraxinus* (3–9%), *Alnus* (3–4%) and *Quercus* (1–2%) reappear after a lapse during the preceding phase, whereas *Ephedra, Taxus, Juglans, Spiraea, Viburnum, Acer* are sporadic and low (under 1% each).

The non-arboreal taxa are chiefly represented by *Polygonum* (3–10%), Rosaceae (2–4%), Cyperaceae and Cheno/Ams (1–3% each) and Caryophyllaceae (2%), whereas Poaceae, *Polemonium* and *Plantago* (under 2% each) are recorded in low values. Aquatic elements, viz. *Potamogeton* (3–4%) and *Lemna* (3%) are better represented than before. Fern spores (monolete 25–55%, trilete 10–20% and *Osmunda* 41–42%) have been recorded abundantly.

In spite of limited sample size, the study provides a synoptic view of the vegetation changes in relation to glacial fluctuations from a glaciated site where very little work has been done because of nonavailability of suitable sediments for both ^{14}C dating and pollen analysis. In this site an alpine-scrub vegetation had occurred prior to 720 ± 90 yr B.P., i.e. around 1200 A.D. in which thermophilous broad-leaved taxa, *Betula, Rhododendron* (Ericaceae) along with herbs like grasses, sedges, Cheno/Ams, *Primula* were the major elements. The thickets of *Juniperus* and *Ephedra* were most probably confined to the rocky habitats. The vegetational composition signifies that the region was under the impact of warm and moist climate during this period. This is also well corroborated by the abundance of fern spores during this phase. The fair amount of pollen of temperate broad-leaved taxa such as *Alnus, Quercus* together with conifers, *Pinus, Abies* and *Picea* during this period could be indicative of the extension of the temperate belt in response to favourable climatic conditions.

Around 620 ± 90 yr B.P., i.e. 1275–1408 A.D., the broad-leaved alpine-scrub vegetation was succeeded by the Juniper-scrubs as evidenced by high values of *Juniperus* and corresponding decline of broad-leaved elements from this site. The non-arboreals comprising grasses, sedges, Cheno/Ams together with ferns also grew meagrely. In general, the poor vegetation cover during this period implies deterioration of climate which was probably colder and drier than today's. Besides, the poor representation of conifers and other tree taxa also suggests that the tree line might have distantly located under the effect of this cold climate.

Thereafter, around 460 ± 100 yr B.P., i.e. around 1401–1487 A.D. and onwards, the summer temperature might have increased again to an extent to support small trees or scrub of *Betula, Alnus, Quercus* and *Rhododendron.* A good amount of pollen of other trees such as *Pinus, Picea, Abies, Cedrus* also indicates that some of these taxa especially *Abies* and *Picea* which grow near tree line had occurred around this site. Decline of *Juniperus* and increase of ferns also indicate less aridity during this period.

The present study based on an analysis of a small number of samples collected from near the snout of Tipra Bank Glacier has provided a broad understanding of vegetational changes *vis-à-vis* glacial fluctuation since around 1200 A.D. In general, two climatic phases, viz. warm moist to cold dry to warm moist again could be recognized in the region. In the beginning, around 1200 A.D. the climate was warm moist and the glacier reached almost to its present position. Around 1275–1408 A.D., however, the climate deteriorated and the glacier might have advanced towards lower elevation. Subsequently, with the amelioration of climate during 1401–1487 A.D. the glacier had started retreating. Presence of several recessive moraines also indicates that the glacier had retreated earlier. In comparison to the global scenario, the climatic amelioration recorded around 1200 A.D. falls within the duration of medieval warm period which is believed to have occurred around 1200 A.D. to 1400 A.D. in the northern hemisphere. Subsequent deteriorations around 1275–1408 A.D. lie within bracket years of climatic changes during Little Ice period. Corresponding to this climatic trend, a climatic amelioration was recorded during 1150 A.D. to 1450 A.D. which reverted to cold and dry again after 1450 A.D.; at Batal, Rohtang Pass, Himachal Pradesh[2].

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Current Science

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The Collected Papers of Albert Einstein – The Berlin Years: Writings 1914–1917. Volume 6. A. J. Kox, Martin J. Klein and Robert Schulmann, eds. Princeton University Press, 41, William Street, Princeton, NJ 08540, USA. 1996. 626 pp. Price: Clothbound $85.

The programme of publishing the collected papers of Albert Einstein has been on since the mid-eighties or so, as a joint effort of Princeton University Press and the Hebrew University, Jerusalem, where most of the Einstein archives are held. This is a massive project expected to finally comprise a total of some forty volumes. Some are devoted to Einstein's voluminous correspondence in various phases of his life, others to his scientific papers divided into significant periods. Along with the so-called documentary edition containing the documents in their original languages, mainly German, a series of invaluable English translations is also being produced by Princeton University Press. Volumes 1, 2 and 3 of the latter have been reviewed in *Current Science*, 1996, **71**, 78 and Volume 5 in *Current Science*, 1995, **69**, 882.

The present volume published in 1996 is the sixth in the Documentary Edition series, and covers the period 1914–1917. This was the start of Einstein's Berlin Years. In early 1912, he had become Professor at the ETH in Zurich, but in April 1914 Einstein moved to Berlin to accept a specially created research professorship, with no teaching duties, at the University of Berlin, under the aegis of the Prussian Academy of Sciences. From the physics point of view this period is most important because the general theory of relativity (GTR) reached its final formulation, and the two fundamental papers on the processes of absorption and emission of photons by matter appeared. This volume has all the original papers in German (except for one in English), and the editors Kox, Klein and Schulmann have added extremely illuminating footnotes, commentary and historical material often bringing matters up to date to present times. It is amusing to notice that many of these papers were published in about a week after submission to the journal concerned – far away indeed from the situation today!

The major part of the contents of the volume deal with the progress of Einstein's ideas on GTR. Three early papers with Marcel Grossmann are concerned with formulations in which the subtle interplay between the mathematics and the physics of general covariance was still far from clear. Indeed in these early papers and up to a few days before the final formulation on 25 November 1915, Einstein had convinced himself that the sought-for field equations for gravitation should allow unambiguous determination of the metric tensor components for given energy momentum tensor as source, and for given boundary conditions. For this reason, the permitted degree of covariance under general coordinate transformations was at various stages limited in different ways – at one point, linear transformations alone; then transformations with unimodular Jacobians alone; and finally imposition of the requirement that the determinant of the metric tensor itself be unity! Later Einstein realized that in some of these restricted versions even rotational covariance would be lost. For these reasons, almost all the earlier papers on GTR (including a review) in this volume are of purely historical value. As one sees the ideas slowly gather strength till they finally break through into transparent clarity, one appreciates the depth of meaning in Einstein's later statement: 'But the years of anxious searching in the dark, with their intense longing, their alternations of confidence and exhaustion and the final emergence into light – only those who have experienced it can understand that.'

At an earlier stage it is amusing to see that after Einstein's inaugural lecture to the Prussian Academy in July 1914, in the discussion, Max Planck wondered why it was necessary to generalize special relativity to include the possibility of accelerated or noninertial observers! Only now may we smile at such incidents.

The first and classic systematic exposition of GTR, Document no. 30, appeared in May 1916; later it appeared as a book as well. The 'summation convention' was used here for the first time.

The full German text of Einstein's first popular exposition of both special and general relativity, completed in 1916 and published in 1917, is given in this volume. This book went through many editions and translations and at various stages Einstein added appendices and extra material in abundance.

The first application of GTR to cosmology, and the introduction of the cosmological constant, date to February 1917, and are reproduced as Document no.43. There are also some short notes on Hamilton's variational principle for GTR. It was only as late as 1931 that Einstein admitted to a change of heart and withdrew the cosmological constant as unnecessary and unjustified.

Around the same period that he was struggling with the formulation of GTR, Einstein also undertook some experiments with Wander Johannes de Haas (son-in-law of Lorentz) to prove the existence of Ampere's circulating molecular currents. More than a century earlier, Ampere had suggested that microscopic current loops were the ultimate sources of magnetism, and Einstein and de Haas set out to prove this by ingenious experiments. While they were in a sense successful, it must be remembered that this was well before the discovery of the spin of the electron and its associated magnetic moment; so later the limitations of the Einstein–de Haas work in the quantitative sense slowly became clear. All the original papers by Einstein and de Haas, and some by the former alone, are included in this volume.

Turning to quantum theory, there are three significant papers. The first two relate to Einstein's persistent attempts to derive Planck's Law in various ways; they introduce his famous A and B coefficients, the concept of stimulated emission (basic to functioning of lasers today) and also the concept of photon momentum. Einstein was very pleased with these efforts since he wrote to Michele Besso: 'A splendid idea on the absorption and emission of radiation has dawned on me'. The third one on quantum theory attempts an invariant formulation of the Bohr–Sommerfeld–Epstein quantization conditions of the old quantum theory – here Einstein was trying to free himself from the limitation to separable and integrable systems, and to express the quantization in a global and invariant manner. It is interesting and amazing that these ideas of his in 1917 find echoes in the dynamics of chaotic systems today.

Other highlights of this volume are

Einstein's first ever political statement – 'Manifesto to the Europeans' dated mid-October 1914 – lecture notes for courses given in winter 1914–1915 on relativity, and notes by a student on the Wolfskehl Lectures given at Gottingen in 1915.

The quality of production of this volume, as one might expect, is excellent. One awaits with anticipation and eagerness the appearance of its companion English translation.

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Physics for Engineers. M. R. Srinivasan, New Age International Publishers, 4835/24, Ansari Road, Daryaganj, New Delhi 110 002. 1996. 519 pp. Price not known.

How much physics should be included in the curriculum of a 4-year undergraduate course in engineering? What kind of physics should be taught, and how should it be sequenced?

These questions are important – not only in their own right, but also because they are relevant to a relatively large segment of our student population. After all, a degree in engineering or medicine is widely regarded in our society as one of the pre-requisites for eternal bliss (the others being a fair bride with a handsome dowry, male offspring, non-stop film music on loudspeakers, and a national cricket team that always wins). They are also questions that have no easy answers. One gathers from the book under review, however, that for students of engineering in Karnataka the study of physics is restricted to a single semester in the first year. The stated aim of the book is to provide a comprehensive text geared to meet this requirement, taking 'the syllabus as a guide for the organization' of the book.

It is at once evident that this places severe constraints on the author. On the one hand, there is his laudable and unexceptionable value system – he urges students to study physics in more detail and depth, with the assurance that 'a proper study of physics would certainly enhance your capabilities as an engineer'. On the other hand, there is the syllabus 'to be covered' – preparing the students to perform the rituals attendant upon the examination system – namely, the appeasement of minor deities named derivations, numericals, short notes, objectives, and so on! The gap between these two poles has become virtually unbridgeable. The author has opted to cater to the second system. Within the parameters chosen, the outcome is a reasonably good book. To understand its limitations, however, it is necessary to understand the two major problems posed by the system, and how they affect even the best of efforts in our textbook scene.

The first difficulty is the inability or unwillingness (or both) of most of our students (and, one might add, many of our teachers) to work out intermediate steps on their own. This has led to the stabilization of a ruthlessly efficient engine: a (vicious) cycle of spoon-feeding and regurgitation in which the working substance is a litany of trivial algebra and arithmetic. Textbooks that do not work out all the steps explicitly are shunned – they are either 'foreign books' or 'foreign-type books' that are 'not suitable for our conditions'. The 'successful' textbooks are poorly printed, excessively bulky hack works that waste a lot of paper and convey little of real substance, let alone challenge the ingenuity or inspire the imagination of the student. Worked out problems most often comprise little more than routine substitution of numerical values in formulas and subsequent simplification in excruciating detail – the argument being that our students only understand material presented in this manner. This is identical to the way our film-makers justify the nauseating trash they purvey as 'what the audience out there wants'. The creation of a vicious cycle in each case is then a mathematical certainty.

The second difficulty is in striking a balance between 'theory' and 'application', especially within the strong constraints posed by a curriculum in which the time allotted to physics is quite meagre. As technology progresses, it becomes necessary to incorporate at least some of the advances into the already abbreviated (4-year instead of the earlier 5-year) BE or B Tech programme (examples: computer-aided design, robotics, . . .). How is this done? Almost always, by removing or reducing portions perceived as unessential, or at least less essential. This is how the 'pure' science part of the engineering curriculum has shrunk over the past 15 years or so – in physics, from a rough average of at least 8 semester-long courses to no more than 3, more often 2, or even 1 (as seems to be the case in Karnataka, and elsewhere too). What happens next in such a situation in tragic: inexorably, what is 'old' gets identified with what is obsolete, and what is new gets confounded with what is relevant. The baby gets thrown out with the bath water: the study of basic principles is eliminated in favour of an inevitably superficial coverage of a patchwork quilt of topics regarded as modern and hence 'relevant' and 'application-oriented', in the fond hope that students who merely hear the magic words or acronyms will automatically become the suppliers of the 'deliverables' of 'futuristic technology'!

It is perhaps considerations along these lines that have dictated the structure of the syllabi of various universities, which the book under review presumably reflects. The sequence of chapters reads: acoustics, nondestructive testing , geometrical optics, interference, diffraction and polarization, fibre optics, laser (*sic*), vacuum science and technology,. . . . This is followed by electronic structure of atoms, semiconductors, dielectric materials, magnetic materials, superconductivity, and *then* magnetism and electricity! Heat and thermodynamics comes next, and then comes the final chapter, basic electronic components – essentially a description of different kinds of resistors, capacitors and inductors. With such a quixotic sequencing of topics, the treatment can only be a largely descriptive listing of facts. Can the average student taking such a course be blamed, then, if he goes away with the impression that physics (which he 'cleared' in his first year by writing short notes on any three out of the following five: noise and its insulation, electron microscope, holography, diffusion pump, energy band theory of crystals (*sic*)) is just a grab bag of disconnected topics like the ones listed above, with little or no unified perspective or guiding principle, half of which can be ignored as it 'was asked last year' and the rest of which is best forgotten in favour of 'real engineering'?!

It is the *system* to which the book under review caters that is under criticism, rather than the book *per se*. Within its limitations, it is an honest effort, certainly better than most other books of its type. The figures are reasonably clear, as is the language. There are not too many places in which trivial algebraic simplification is written out in detail. The author has taken care to avoid errors and misinformation to the extent possible – although there are passages that either beg the question (example: Sec. 16.5 on the zeroth law of thermodynamics) or are too obtuse to be of any help in understanding the matter under discussion (examples: Sec. 8.6 on holography, Sec. 11.18.1 on light emitting diodes). On the whole, one may regard the book as a mild form of medicine that may alleviate some of the symptoms of the malady pervading the system, without attempting to cure the illness itself.

But is there no satisfactory solution to the dilemma itself – namely, the problem of striking a balance between basic principles and applications in a highly time-constrained curriculum? This reviewer believes that there does exist a reasonable solution (modulo adjustments of detail depending upon the actual circumstances). When one has to make an absolute choice between a basic principle that has very wide applicability, and a specific application whose technical details are in any case too complex to be dealt with satisfactorily in an elementary course, the correct choice is virtuous and clear! A careful exposition of the former, with the help of simple illustrations, would stand the student in good stead in his subsequent career, both educational and professional. To paraphrase a thoughtful colleague's remark, the student would then have been empowered rather than bedazzled, and that's a good thing to be.

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Ecology and Sociobiology of Indian Primates. M. D. Parthasarathy, Dynaram Publications, 20, 1st Floor, South Cross Road, Basavanagudi, Bangalore 560 004. 119pp. Price: Rs 100, $10.

It is a small monograph on the ecology and sociobiology of some Indian (rather south Indian) primates, written lucidly after proper scientific understanding of primates. The monograph, appropriately illustrated with self-explanatory line drawings, provides a unique overview on many hitherto unknown social behaviour in primates. It has covered the different categories of behaviour and provides a detailed account on loris, the bonnet macaques, lion-tailed monkey, Hanuman langur and Nilgiri langurs. The book provides a synthesis of the most current information and theoretical significance. The language used is not very difficult or complicated even for a learner primate researcher. It is a monumental piece of work which will be of immense interest and use to diverse readers specially for researchers, the academic community and scholars alike for comprehending primate eco-ethology. In my opinion, it is a commendable job by M. D. Parthasarathy which will guide young ethologists.

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Atlas of Paleogene Ostracodes of Rajasthan Basin. ***Paleontol. Indica*, No. 4.** Anil Bhandari. KDM Institute of Petroleum Exploration, Dehradun 248 195. 157 pp. Price not known.

For the first time there has been a sincere effort to prepare an atlas of Paleogene ostracodes of Rajasthan basin. An exhaustive study of the surface outcrop samples and subsurface well samples (from 5 wells) were examined by the author. One hundred and twenty four species of which eleven taxa are in open nomenclature are included in the work, illustrated excellently in the plates.

Ostracodes are mainly utilized for environmental interpretations and their scope for dating of rocks is limited due to their benthic habitat and strong response to environmental changes. The author could have included a chapter on environment in this publication.

The author has contributed an excellent work more of which must be coming in the near future. The data available from different sedimentary basins are enormous and dedicated workers in ONGC must contribute to this field. Workers in India and abroad are awaiting anxiously for more atlases and if possible, a stereo-atlas similar to that published by Leicester University, UK. The stereo-atlas will clear the confusions in taxonomy, since examining the stereo-pairs under ordinary stereoscope is easier than going through actual specimens or holotypes, etc., Bhandari's work is highly appreciated and will benefit a lot of research workers in this field.

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Pancheti Koteswaram – An obituary

P. K. Das

With the passing away of Pancheti Koteswaram (1915–1997) on 11 January this year, our country has lost an eminent meteorologist of the postwar period. His best contributions were in synoptic meteorology and practical weather forecasting for which he will be remembered by his colleagues. His ability in these fields was coupled with an innovative approach to emerging areas of new technology. He had a visionary outlook for the growth of Indian meteorology.

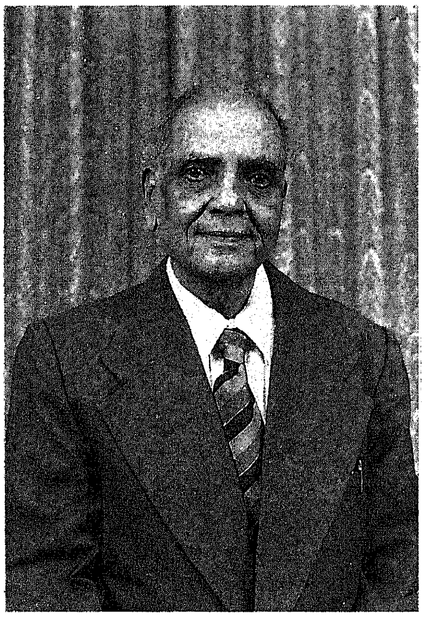
Koteswaram was born on 20 March 1915 at Nellore, a small town in coastal Andhra Pradesh. In later life he would often tell us about a cyclone which struck Nellore in 1927, when he was a boy of twelve. This, he said, generated a lifelong interest on tropical cyclones.

His early education was received at Nellore. In 1931, he was placed first in the Intermediate examination of Andhra Pradesh. The Sir R. Venkataraman gold medal was awarded to him by Andhra University for this achievement. Later he joined the Presidency College at Madras from where he graduated in 1934 with an Honours degree in Physics. Thereafter, he worked in junior academic positions at different colleges of Andhra Pradesh. Around 1936 he embarked on a research project on the Raman effect, under the guidance of T. Ramakrishna Rao. He received his doctorate degree (D Sc) in 1939 from Madras University. His thesis was on 'Studies of associated liquids by the Raman effect'.

Koteswaram joined the India Meteorological Department (IMD) in August of 1940. The early part of his career was spent on operational weather forecasting at different meteorological centres of India. Interestingly, one of his early postings was at the Cyclone Warning Centre at Alipore, in Calcutta. Here he acquired the basics of cyclone prediction and warning.

I was introduced to Koteswaram in 1949, when I joined the Meteorological Department at Poona (now Pune). I was impressed by his command over data and data-related problems. Around this time Koteswaram become interested in the upper air over India. This led to his discovery of an easterly jet stream over peninsular India. Its appearance provided Indian forecasters with a valuable tool for detecting the arrival of the summer monsoon.

As mentioned earlier, Koteswaram's other area of interest was on tropical cyclones. Once a cyclone was detected, he would follow its path and predict its future with meticulous care. I remember spending many hours with him debating the observations on a weather chart. He loved a debate, but like Voltaire, the French philosopher, he would often end by saying: 'I differ from you emphatically, but I will defend your right to differ till the last drop of my blood'. Differences of opinion seldom led to any permanent rancour.



As a sequel to his work on tropical cyclones and the easterly jet stream, there were invitations for him from many centres abroad. He went to the University of Hawaii in 1961 as a research scholar. Similar assignments were provided for him by the University of Chicago and the National Centre for Atmospheric Research (NCAR) in the USA.

Koteswaram was elevated to the top position of the Meteorological Department, the Director General of Observatories (DGO), in 1969. He was due to retire in 1973, but his work earned him a two-year extension up to March 1975. The Government of India awarded a 'Padma Bhushan' to him in 1975. After retirement, he worked as a consultant with the government of Iran up to 1977. Subsequently, he settled down at Waltair as an Emeritus Professor of Andhra University, and whenever a cyclone was brewing near Andhra Pradesh he was always present at the nearby meteorological office to watch its movement. He named his house in Waltair 'Varsha' (rain), which was not surprising when we consider his love for the atmosphere. 'Varsha' was a pleasant meeting place for his students and colleagues because of the warm hospitality of Koteswaram and his family.

During his tenure as the Director General of Observatories, Koteswaram introduced several new ideas and innovations. One of his early contributions was to improve the quality of upper air data. His initiative led to a uniform design for the radiosondes used in India. Automatic Picture Transmission (APT) facilities from the US Weather Satellites were introduced by him. Meteorological radars in the X-band (3 cm) and the S-band (10 cm) were also introduced. As these radars were manufactured by Bharat Electronics Ltd., Koteswaram tried to make the country as self-reliant as possible in the then emerging area of radar meteorology.

True to his abiding interest in tropical cyclones, Koteswaram took vigorous steps to introduce a close network of cyclone detection radars along the east and west coasts of India. Along with the APT facilities, these innovations did much to improve cyclone detection and prediction in India. Developments in instrumental technology were followed by cyclone warning centres in each of the coastal states of our country. An annual meeting of the Cyclone Review Committee also helped to keep meteorologists aware of the latest developments in this field.

India played a leading role in international collaboration during Koteswaram's time. He was largely instrumental in setting up a Regional Meteorological Centre (RMC) and a Regional Telecommunication Hub (RTH) at New Delhi. These centres were a part of World Weather Watch (WWW), a global project that was launched by the World Meteorological Organization (WMO) to improve the collection and analysis of data at the principal meteorological centres of the world. Koteswaram was also closely associated with the International Indian Ocean Experiment (IIOE) of 1959–1965. For his constructive role in the different WMO projects, Koteswaram was elected a member of the Executive Committee of WMO. Later, he was made the Third Vice-President of WMO. He was the first Director General from India to receive this honour.

Outside his professional activities, Koteswaram was a fun-loving personality with a keen sense of humour. He loved a light-hearted joke, even sometimes on himself. His humour often enlivened a dreary discussion. This made life much easier for others.

He died after a brief illness and is survived by his widow, four daughters and a son.

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Erratum

Solar eclipse and neutrinos (*Curr. Sci.*, 1996, **70**, 848–849)

Mohan Narayan, G. Rajasekaran and Rahul Sinha

The neutrino emerging from the sun is an incoherent mixture of mass eigenstates and hence the correct quantum mechanical calculation of the enhancement during the eclipse is different from the classical treatment of eqs (2) and (3). Although the corrected numerical results are similar to those in Figure 1, the enhancement now occurs at still smaller values of δ and these will be presented in a detailed paper.

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Physical Research Laboratory,
P.O. Box No. 4218,
Navrangpura, Ahmedabad 380 009.

In addition, the candidate should arrange for at least two letters of recommendation to be sent directly to the above address. The last date for receiving the completed application is 30 May 1997. Candidates called for interview from outstations will be paid to-and-fro first class train/bus fare by the shortest route from the address given in the application.

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Date: 17–21 May 1997
Place: Mumbai

IIIrd Continuous Medical Education in Haematology is oriented towards PG students and senior staff members from pathology, paediatrics and medicine. It includes both diagnostic and therapeutic aspects.

Contact:
Dr M. B. Agarwal
Programme Director
107, New Wing, Bombay Hospital
12, Marine Lines
Mumbai 400 020
Tel: 022–4144453
Fax: 022–4140058

Training Programme in Methods to Evaluate Mammalian Cell Survival and Cytogenetic Changes

Date: 16–28 June 1997
Place: Manipal

It includes the common techniques in mammalian cell studies.

Contact:
Prof. P. Uma Devi
Department of Radiobiology
Kasturba Medical College
Manipal 576 119

Contact Course in Isotope Geology and Geochronology

Date: 14–22 July 1997
Place: Ahmedabad

The course is designed to suite University/College teachers, research scholars and young scientists in the field of earth sciences.

Contact:
Dr Kanchan Pande
Course Co-ordinator
Earth Sciences Division
Physical Research Laboratory
Navrangpura
Ahmedabad 380 009
Phone: 079–462129
Fax: 079–6560502
E-mail: kanchan@prl.ernet.in.

II Congress of Federation of Indian Physiological Societies

Date: 15–17 September 1997
Place: Tirupati

Topics include: Physiological basis of biotechnology; Neuromuscular physiology and muscle disorders; Enzymology – pure and diagnostic; Metabolic and endocrine disorders and clinical diagnosis; Applied physiology and economics; Behavioural physiology; Pharmacophysiology and immunology; Development physiology, reproductive physiology and gynaecology; Environmental physiology; Space physiology; Non-mammalian physiology; Physiology of domestic animals.

Contact:
Prof. P. Venkateswara Rao
Chairman
Organizing Committee
II Congress of F.I.P.S.
Department of Zoology
Sri Venkateswara University
Tirupati 517 502
Phone: 0091–08574–24166
Fax: 0091–08574–27499/29111/25211
E-mail: nicnet.svims.nic.in!root.

Training Course in Geological Mapping in Volcanic Terranes (Deccan Traps)

Date: 3 weeks from 10 September 1997
Place: Nagpur

Geological Survey of India (GSI) Training Institute is organizing a training course sponsored by Department of Science & Technology (DST), under the SERC Winter School Programme. The course will consist essentially of field training with due complement of theoretical lectures. The course is opened to Research scholars/Lecturers from universites/colleges and professional geologists from Central and State Government Organizations and Institutions.

Contact:
Deputy-Director-General
GSI Training Institute
Bandlaguda
Hyderabad 500 068
Phone: 4022681
Fax: 4022680

CURRENT SCIENCE

A fortnightly journal of research

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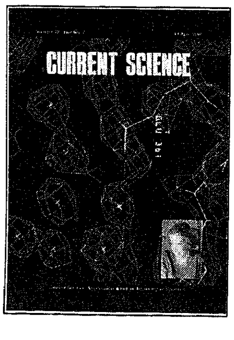

COVER. Electron density map of the steroid binding pocket of cholesterol oxidase complexed with dehydroisoandrosterone (see page 477). *Inset*: Dorothy Hodgkin aged about 20.

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In this issue

Dorothy Hodgkin

The articles in the special section (**page 447–489**) on Dorothy Hodgkin in this issue are based on a one-day symposium held in her memory on 12 July 1996 at the Royal Society, London, organized by the British Crystallographic Association. The occasion marked the formal release of the *Collected Works of Dorothy Crowfoot Hodgkin* published in three volumes by the Indian Academy of Sciences. This issue of *Current Science* also reproduces the text of introductory articles in the *Collected Works* which provide a wide ranging view of her impact on crystallography worldwide.

I had the rare privilege of editing the *Collected Works* and the even greater privilege of having known her for three decades. This may be the place to look back upon my association with her*.

Seeking Dorothy

The *Bible* says seek and you shall find. Yes, I sought Dorothy since the early 1950s, when I was charged with and accepted the responsibility of building up a school of X-ray crystallography in Bangalore; But I found her only a decade later in 1964. When I had read most of her papers in the fifties I felt that she was foremost amongst those who were changing the face of X-ray crystallography, in that she was solving structures of substances whose structures were quite unknown, thus elevating the very status of the science of crystallography. I felt she could be the salvation of crystallography in India if Indian crystallographers could hitch their wagons to her. I longed that my students after obtaining their Ph D degrees in India could work with her, get inspired by her, come back to India and contribute, if possible, to the new wave in crystallography she had started.

Strangely enough and to my utter surprise I found that Dorothy had heard of me and my struggles in India with multiwavelength anomalous scattering techniques. She told me that she had heard of me from the late Gopinath Kartha (who met her in Oxford on his way to USA to work with David Harker on ribonuclease) and also, as far as I was concerned, most unexpectedly from the late N. V. Belov, the renowned Soviet crystallographer, who had met me in India in the fifties. She sent some gentle enquiries as to whether I could spend a year or two with her trying out the anomalous scattering methods in larger molecules, particularly in proteins like insulin. I informed her that she could do much better by offering a fellowship to my student K. Venkatesan (Van) who was just finishing his post-doctoral year with Jack Dunitz in Switzerland. This I thought could be the thin end of the wedge by which my students could join Dorothy and she too could have an opportunity of judging their quality and that of the Bangalore crystallographers. Dorothy did offer Venkatesan a fellowship. It was a successful experiment because since then 7 or 8 of us from Bangalore have worked in Dorothy's laboratory in Oxford and have tried to imbibe her spirit and culture of crystallography. I understood that Jack Dunitz himself strongly recommended Venkatesan to Dorothy for which I shall always be very grateful to him.

My going to Oxford to be with Dorothy is another story. I conjecture David Phillips (now Lord Ellesmere), who gave the beautiful inaugural lecture in the symposium, played a vital role in it. He came for a conference convened by G. N. Ramachandran in Madras. I was then at IIT Madras. As soon as David came he told me that he would like to have a long chat with me. I suspected that he had come to meet me on behalf of Dorothy. On a free day I arranged for David and myself to go to Mahabalipuram, one of the gorgeous beaches close to Madras. We had a long chat there. I feel that he must have given a good chit about me to Dorothy because a few days after his return I got a letter from her inviting me to Oxford offering me a senior fellowship saying that she would also need my help for looking after her Ph D students when she would be away in Ghana with her husband Thomas. I was greatly flattered with Dorothy's offer and I accepted it with alacrity and thanked my stars.

Looking after Dorothy's students was really no problem at all for they did not need any looking after. During my stay in Oxford I had the pleasure of seeing a group of young talented crystallographers flowering. Every week each one of them would come and tell me what they had done. I put on a wise look and made a few comments and I was told later by Dorothy that they were very useful to them. I also found that often the problems that were being tackled in Dorothy's lab by her students were indicative of her innovative thinking. For example, one of the problems was solving the structure of a comparatively small molecule in a crystal that displayed non-crystallographic symmetry. The solving of the crystal structure itself was by no means difficult but I feel what Dorothy had in mind was to investigate whether, after refining the structure, any phase relationships emerged due to the presence of the non-crystallographic symmetry. Dorothy's lab was one of the earliest ones to combine the photographic method and a computer-controlled microphotometer for the quick collection and indexing of crystallographic data. She foresaw that photographic methods may probably finally replace diffractometric ones, especially when data on hundreds of thousands of reflections had to be collected, indexed and their intensities measured. This work was done in her laboratory long ago and in my view paved the way for the later sophisticated methods which were developed afterwards and which are now in vogue. Dorothy's lab was amongst the earliest to cool protein crystals to reduce their being denatured by X-radiation. She was amongst the earliest to use computers for solving the structures of large molecules.

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*Based on an after dinner speech delivered at the Royal Society, London on 12 July 1996. The speakers at the meeting included Lord Phillips of Ellesmere, Prof. David Blow, Dr Bill Duax, Dr Peter Roach, Dr Phil Evans, Prof. Jenny Glusker, Prof. Tom Blundell, Prof. Guy Dodson, Prof. Jack Dunitz, Prof. Michael Rossman. Sessions were chaired by Prof. Louise Johnson, Dr Eleanor Dodson, Prof. Bob Williams, Dr Margaret Adams, Dr Max Perutz.

New phasing techniques

Dorothy was always looking out for new techniques of phase determination which she could apply in her work. It was only recently that I knew from David Sayre that it was Dorothy who invited him to Oxford to develop his now-renowned triplet method. When the Patterson synthesis came out she adopted it and mastered it. One has to read her Patterson Memorial lecture to know about her views on this subject and how she used this technique all her life. When the Bijvoet method of phasing using anomalous scattering was published she got into it heart and soul and tried it out on two fairly large non-protein molecules – cobyric acid and monocarboxylic acid of Vitamin B_{12}, which were in my view landmark structures to be solved *completely* using *only* anomalous scattering methods. Dorothy was obviously building stepping stones for her to use the Bijvoet method for solving the insulin structure. Here again many innovative techniques for using anomalous scattering were developed in her laboratory. For example, in zinc insulin, HgI_4 was introduced to see whether a cluster of anomalous scatterers would be of use in phasing. It was shown that it was not so useful. Also, new techniques were evolved for calculating the contributions of the heavy anomalous atoms alone, using simultaneously, anomalous and isomorphous data. This definitely played a major role in the final solving of the insulin structure. At the symposium Max Perutz has described in his inimitable manner how Dorothy attracted his attention to Bijvoet's suggestion of using the multi-isomorphic technique for phasing and how she even berated Max for not having picked this out from Bijvoet's famous 1951 paper and in having lost time in not immediately commencing using this method for solving the haemoglobin structure. We all know that the final solution of the structure depended much on this method.

What surprised me greatly was that as early as 1964–65 Dorothy discussed with me the three-beam interferometric method of using Renninger reflections suggested by Lipscomb and Fankuchen (1949), and earlier by Bijvoet and Macgillavry (1939). Most practising crystallographers would not even have heard of the method at that time. She, however, knew of the valiant effort Ben Post was putting in to make the method work which he finally perfected only in the late seventies. When she asked me whether this interferometric method could be used in the case of proteins, my reply at that time (1964–65) was 'If Renninger reflections could be observed in protein crystals then there was no reason why it could not be used. However I felt that the experimental methods involved may prove quite formidable'. I still do not know whether my answer was correct or not, but this method is now being pursued by many like Weickert, Hummer and others and they seem to have succeeded in determining the phases of a few intense reflections in lysozyme.

When anomalous scattering was discovered also in neutron scattering, Dorothy asked me to investigate whether this could be applied in the phasing of very large molecules. When I found there was a possibility of doing this by putting in an anomalous scattering isotope into the structure and using the appropriate neutron wavelength, she immediately got insulin crystals doped with the required cadmium isotope and put them in the neutron beam of the Harwell reactor. This was in spite of the fact that her mentor, the great J. D. Bernal, had told us both categorically that while the idea was basically correct the intensity of the neutron beams from the then existing reactors may not be sufficient to give any definite result. As expected, Dorothy's experiments proved to be unsuccessful. But to my mind this was of no consequence; it just showed the spirit with which Dorothy pursued new ideas.

Since 1933 Dorothy has been writing long critical reviews of crystal structures (see *Collected Works of Dorothy Crowfoot Hodgkin*, Vol. 3). The first one was in 1933, a 52-page article. In this she says, 'The methods of crystallography have not radically changed but in the past two years they have acquired additional certainty and precision. Fourier analysis which is so laborious has been undertaken in a few laboratories.' These reviews by her are so thorough and so critical that even the scientists who solved the structures would have gained much by reading her reviews. I feel that it was during this process of study for writing these reviews that she acquired her intimate knowledge of inter-atomic distances and bond angles in molecules under different conditions as also molecular configurations and orientations which proved invaluable in her later work.

In another review she welcomed the Patterson technique and explained it in some detail. She applied it almost immediately in its three-dimensional version for solving the structure of cholesteryl iodide. I remember learning all about the intricacies of the Patterson technique from her clearly written paper. She made it look so simple. She admonished crystallographers for not sharpening the Patterson maps which gives much more information and is much easier to interpret. She was a master of the Patterson synthesis. I am reminded of Max Born saying of C. V. Raman, 'His mind leaps over mathematics'. This could apply to Dorothy as well. Dorothy could deconvolute any Patterson map in her mind and deduce exactly where the atoms should be situated.

As soon as I came to Oxford I had the unique honour of sharing for three weeks the same office as Dorothy; indeed I also shared a part of the extra large table she used. I sat on the other end watching her work. When she worked her concentration was incredible. Nothing or no one could distract her. She always lightly hummed a tune and *invariably* had a light smile on her face when working on a structure. She held a molecular model in one hand and sheets of Fourier maps made using approximately estimated phases and sheets and sheets of 3D sharpened Patterson maps in the other. She used to pick up the model and the Fouriers and the Pattersons and study them most carefully to derive the positions of the atoms. To do all this it would appear as though she needed more than two hands and that is why I was very impressed with Maggie Hamblin's insight in the painting of Dorothy – a picture which is now in the National Portrait gallery – in which she has represented Dorothy with many arms and she looks almost like one of the many-armed fierce goddesses of the Hindu Pantheon. We are told by those who know these matters, that goddesses are created by the Almighty to do a specific job which normal human beings cannot accomplish. In the case of Dorothy it was obviously for solving structures which most mortal crystallographers could not. But one aspect of this portrait I do not like is the fierce face Dorothy has been given. Dorothy's face was always calm with a smile. It always radiated peace and tranquility.

Suddenly the humming would stop and she would have a beatific smile on her face. This was indicative of her having plucked out a few atoms from the entangled web of Pattersons and Fouriers and placed them in their right position in the structure.

Now a few words about Thomas Hodgkin. One must read Dorothy's biographical and historical papers which are published in the *Collected Works* to see how much study she puts in to write these. They are filled with quotable quotes. In the biographical memoirs of Kathleen Lonsdale Dorothy quotes, 'For a woman to become a first class scientist she must first of all choose a right husband.' Dorothy no doubt chose extremely well. I knew Thomas Hodgkin very well. We became good friends and we spent hours together conversing and arguing. He had a sharp and a quick tongue but there was absolutely no malice in him. He was a scholar with a deep knowledge of history and the classics – also languages like Latin, Greek and Arabic. He had a remarkable sense of humour which could keep everyone laughing most of the time.

In preparation for a later story I must mention here that both husband and wife had the disconcerting habit of falling asleep when one was talking to them, thus sapping away much of one's self-confidence. When Dorothy went to Stockholm to receive the 1964 Nobel Prize, Thomas also went. In one of the Swedish newspapers there was a picture of Jean Paul Sartre who had refused the Nobel Prize and under the photograph there was a sub-title 'He was not there'. Next to it was another photograph with the legend 'Neither was he'. It was a beautiful and clear picture of Thomas soundly asleep during the prize-giving ceremony.

When Dorothy came to India as a Raman Visiting Professor, Thomas accompanied her. Unfortunately the Bangalore weather did not suit him and his asthma and emphysema worsened and he fell seriously ill. We continually telephoned his doctors in Oxford who told us that on no account must he be moved and there must be no jerky motions at all as his frail lung tissues may collapse at any time. We took all the requisite precautions. I will not tell you the story how as we could not move him into the clinic, as it were the mountain went to Mahomet; in that a big X-ray machine was brought from the clinic to the IISc guest house to take the X-ray photographs of Thomas' lungs. After sometime I noticed that Dr A. R. Pai, our doctor in attendance, after giving all the requisite medication was chanting *mantras*. He told me that all the possible efforts by human beings had been made and it was time to seek divine intervention and he requested me that I should promise to pay rupees thousand to the god of the Seven Hills, Venkateswara of Tirupati if Thomas survived this attack. I told Pai that I did not believe in all this. Pai's reply was exactly the one Neil Bohr gave when he was asked about the horseshoe hanging in his office – 'it works even if you do not believe in it'. Fortunately for all of us, Thomas became well and Pai asked me whether I had sent in the thousand rupees to the temple of Lord Venkateswara. When I said I had not, Pai said, contract is a contract, even if it is made in one's mind and so I must send rupees thousand immediately to the Tirupati temple, particularly as the party of the second part had kept up his end of the contract. So without further ado I sent a demand draft of rupees thousand to the temple at Tirupati*.

In 1982 we got a letter from Dorothy saying that Thomas had died. It was sad. When we saw her next, there was a change: something was missing, Joy had flowed out of her and she appeared far more pensive and serene.

Dorothy often said that she greatly regretted that the four persons – P. P. Ewald, J. D. Bernal, A. Patterson and J. M. Bijvoet were not awarded the Nobel Prize, as they were the persons who started it all and cut new and successful pathways in X-ray crystallography. She also was keen that the Pugwash organization should also be honoured by a Nobel Prize for peace. (This did happen in 1995.)

I have often been asked when I got the idea of publishing Dorothy's Collected Works. I remember the exact moment when this happened. When Dorothy and I visited North-Bengal University, in Siliguiri we were close to the foot hills of the Himalayas. Dorothy who never asked anything for herself said that she would like to view the Himalayas and see at least some of the high peaks. We went up to Darjeeling in a hired ramshackle car. Dorothy could not stand even the heights of Darjeeling and was quite sick and I with foolhardiness did not cancel the visit. Early in the morning we stood on Tiger Hill and saw the sun before it rose, picking up at some of the highest peaks starting with great Mount Everest. Finally, we saw it rise and the Kanchanjunga appeared as a pyramid of gold bathed in the golden sunlight in front of us. It was one of the most magnificent sights I have ever seen, comparable only to two of Natures grandest spectacles I have seen, the Grand Canyon in the US and the Victoria Falls in Africa. It was at that time standing at Tiger Hill along with Dorothy, when she was viewing and surveying the high peaks of the Himalayas and I was seeing Kanchanjunga that I got the complete import of the sentences often repeated by the late S. Chandrasekhar, the renowned Chicago astrophysicist:

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*There is a foot note to this story. When Thomas was passing through the crisis Dorothy had to give the inaugural lecture at a symposium. She appeared at the proper time, and reportedly gave a memorable lecture. No one would have guessed that she had just left her husband fighting for his life in the guest house just a kilometre away.

> *'The pursuit of science has often been compared to the scaling of mountains, high and not so high. But who amongst us can hope, even in imagination, to scale the Everest and reach its summit when the sky is blue and the air is still, and in the stillness of the air survey the entire Himalayan range in the dazzling white of the snow stretching to infinity? None of us can hope for a comparable vision of nature and of the universe around us. But there is nothing mean or lowly in standing in the valley below and awaiting the sun to rise over Kanchanjunga.'*

When I was with Dorothy on Tiger Hill, I suddenly got the idea that budding young crystallographers of the world may gain much if at least some of the *selected papers* of Dorothy could be published. I did not ask her permission then. Six years later in 1985 when I went to her home, Crab Mill, in Ilmington I asked her and her answer was a categorical *no*. Dorothy said that she had so much work still left to do. She also said that if I took up this project I would ask her to write the introduction which she could ill afford to do at that time. Later in 1991 I again visited her in Ilmington. She had had a fall and had broken her hip-joint and she was immobile and confined to a wheel chair. She was a pathetic sight. One felt extremely sorry for her. She looked so fragile and the main discussion then with the health workers was how to give her a bath. When this engineering problem was solved she looked at me with a smile and said, 'I think you could now go ahead and publish my Collected Works.' I was extremely happy. Almost immediately she said with another charming smile that she would be leaving in a day or two for the Lindau conference of Nobel Prize winners. I was surprised and shocked and wondered how she could do it in her state of health. But she did go in the wheel chair along with her niece. One could not but admire her courage and her indomitable spirit.

It is said that one does not become a great scientist by tackling insoluble problems but scientists like Rutherford and Dorothy who changed the very face of science did take up problems which to most were obviously insoluble, but by their sheer genius they solved them. In this sense they justified Peter Medawar's definition of science as 'the art of the soluble'.

S. Ramaseshan

Understanding the sun

The sun has been held in awe and wonder in the human mind from time immemorial; all along the course of development of knowledge, many attempts have been made to properly understand this heavenly object. The early telescopes employed by Galileo spent considerable time for solving the mystery of the strange markings on the sun; remarkable periodicity which was noticed in their appearance had opened up a series of questions, which continue to the present day. Observations through the ages by employing numerous techniques and devices continued bringing out a flood of new information, keeping scientists busy in unfathoming the mystery of this heavenly splendour.

The pathway to gathering the data has not been smooth; there have been many hurdles in this task. The day-night cycle at any observatory has kept the sun hidden from view for half the time. To circumvent this limitation, scientists have established chains of observatories all around the globe; but still the Earth's atmosphere has always prevented an unhindered view, by disturbing images and absorbing bulk of radiations which bring messages from the sun. Finally an observatory has been floated in space, far out of our planet which now provides a continuous, unrestricted view of the sun all the year round. An account of this unique observatory SOHO, and some question for which answers are awaited appear in this issue (**page 437**).

J. C. Bhattacharyya

Subhas Mukerji

Subhas Mukerji was responsible for the birth of India's first 'test tube baby' in October 1978. Three years after the event, he ended his life in 1981. In the intervening period, the medical establishment aided by an uncaring and unresponsive government machinery, instituted enquiries conducted by committees with suspect credentials, used the weapon of 'transfer' and eventually discredited Mukerji and drove him to his end. Many years after the event, T. C. Anand Kumar (**page 526**) analyses Mukerji's scientific work and comes to the remarkable conclusion that Subhas Mukerji was indeed the 'architect of India's first test tube baby'. Anand Kumar is in fact credited with being the leader of the team that orchestrated India's first 'official test tube baby' in 1986. The disheartening tale of Subhas Mukerji should make us pause to reflect on the need for balance and reason in assessing scientific claims. Anand Kumar's essay, however, reassures us that the tradition of scholarship is still alive and well.

P. Balaram

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CORRESPONDENCE

Of politicians and theologians: The post-Dolly scenario

As usual, the conclusion seems to be that, where evil is concerned, humans seem to have a monopoly.

—Colin Wilson

History is witness that all great scientific achievements have usually been followed by frenzied and irrational doubts, usually expressed by political and/or religious leaders – the self-appointed guardians of our society. The alchemists of old, who laid down the foundations of modern chemistry were hounded (and also cultivated according to the need of the hour) in the medieval period. Copernicus was persecuted for propounding the theory of a heliocentric universe. When Edison invented the carbon filament lamp in 1879, William Preece informed the House of Commons that the concept of electric light in homes was fanciful and absurd. For fear of the Inquisition, Leonardo da Vinci spent several years secretly designing prototypes of flying machines which uncannily resemble the helicopters of today. More recently, computers and robots were viewed with suspicion and fear that these would eventually take over the world as rulers of mankind. However, anyone, who has been even remotely associated with computers and robots will be well aware as to how fragile and dependent these machines are on humans for their operation and maintenance.

When the world woke up to the birth of Dolly, a cloned 'true-to-type' sheep at the Roslin Institute, Edinburgh, several scientists and leaders of different hues issued statements for and against such scientific researches. As the Roman Catholic Church has long expressed its opposition to any form of reproduction divorced from the sexual act, the Vatican, in a knee-jerk reaction, issued a statement that every person has the right to be born in a human way and not in a laboratory. Several persons in the world today, technically known as test-tube babies therefore, have no right to exist because they were conceived in test-tubes instead of a human womb. Although, taking the medico-legal aspects into consideration, they were born in a human fashion, only conceived artificially.... The arguments are endless.

As any primary student of biology knows, the birth of a new organism is not confined to the sexual union of gametes alone; vegetative propagation, asexual reproduction and even parthenogenesis are common phenomena in the world of biology. From simple cross-breeding and grafting to somaclonal hybridization and oligonucleotide-directed *in vitro* mutagenesis – the twin sciences of genetics and molecular biology have evolved with such gigantic steps that creating either a biotype or an identical clone has become a routine affair rather than a source of wonder. 'True-to-type' plants have been produced by tissue culture for the past several years. Oncologists have been using cloning techniques to study the origin and growth of cancer cells. Then why the hullabaloo if a true-to-type sheep or a couple of simians have been produced through cloning techniques?

If the phenomenon of giving birth to a new being is a Divine Act, it should not be confined to mammals alone but should also be applicable to the plant kingdom and lowly invertebrates. While the author is no authority on theology or metaphysics, it is apparent even to a layman that God should not be defined according to the limited vision of our religious and political leaders. Scientific inventions and discoveries should not be looked upon as a threat to God's existence but as another manifestation of His Divine Presence. Satellite TV and space travel by humans do not imply that God is dead. One need not revert to the Stone Age, simply to acknowledge the existence of God.

The US administration has issued a directive, banning the federal funding of research on cloning of human beings. Is it really justified? Can we arrest the progress of science with a political ban? Can important and significant scientific discoveries simply be wished away? It is unfortunate that the world did not act with equal alacrity to ban nuclear weapons of mass destruction. It appears that we are heading towards the Middle Ages where scientific ideas not in consonance with the limited vision of politicians and religious leaders were banned and the scientists put to death.

Coming back to Dolly, Steven Spielberg, in his sci-fi magnum opus, *Jurassic Park* had envisioned the re-birth of dinosaurs through cloning. The public lapped it up with delight as it had lapped other sci-fi fantasies in *2001 – A Space Odyssey*, *Demon Seed* and *Star Wars.* However, while the public is relatively calm and scientists delighted with the immense possibilities of cloning techniques, it was left to our political and religious leaders to stir up a controversy and contribute to mass hysteria. Perhaps for publicity, if not for votes.

It has been truly said that more powerful than a thousand armies is an idea whose time has finally come. Just as by banning a book, one cannot ban the idea behind it; by banning researches on cloning in humans, one cannot ban the immense possibilities behind such technology. Moreover, it should be remembered that cloning is the biological equivalent of photocopying. To use a crude analogy, any layman can photocopy a Shakespearean sonnet from a book but it takes God in His munificence to produce a Shakespeare in a millennium. However, we are digressing. Perhaps the actual fear is not of cloning technology *per se* but the apprehension that the technology may be misused to produce a race of 'superior' Anglo-Saxons, lending an almost prophetic touch to Colin Wilson's observation.

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Biosafety of transgenics

I have read with interest the article 'Transgenic plants and biosafety concerns in India' by P. K. Ghosh (*Curr. Sci.*, 1997, **72**, 172–179). It brings out all the issues that have been raised regarding the biosafety of the transgenic plants, and the procedures adopted in India for the clearances required at various levels for developing, evaluation, upscaling and commercial cultivation of transgenic plants. The latter information should be extremely useful for the researchers working, or intending to work, in this area, especially the young research scholars, who develop such plants while working abroad and wish to bring transformed cell cultures or plants to their own laboratories back home. However, while reading the article it was felt that it only echoes the concerns that have been raised[1–3] but fails to bring out the widely accepted viewpoint of the plant geneticists and breeders especially in North America, Organization for Economic Co-operation and Development (OECD), China and Australia where, as stated by Ghosh, transgenic crops will be grown over an area of over four million hectares this year. Most of these countries, in the past, have shown greater concern for the environmental issues than in India.

Rissler and Mellon[1] argued that (i) The r-DNA methods are more powerful tools for genetic manipulation than the conventional methods used by the breeders, so far. (ii) Prior to the r-DNA methods, genetic manipulations were confined to the use of genetic variability in the primary, or at best, the secondary gene pool of the crop species. (iii) With the new techniques, genes of a large variety from any living organism or a synthesized gene can be expressed in plants, while the conventional methods were limited to replacing alleles or their deletions.

They therefore inferred that r-DNA methods may be linked with 'greater unpredictability' in comparison to the conventional methods, which are limited to genetic manipulation at cellular level alone. In this context, some of the decisions of the National Experts on Safety in Biotechnology of the OECD[2] are very pertinent. They have recognized that 'the safety of an organism is independent of the process of genetic modification *per se*. It is the characteristic of the organism, including new traits, (however introduced), the environment and the application that determine the (likelihood of) risk of the introduction.' Thus, the environmental risks from the transgenic crop plants also apply to the cultivars with similar properties developed using the conventional methods of plant breeding. An insect-resistant crop cultivar developed using inter-cultivaral hybridization or distant hybridization poses the same risk as the one developed by using the genetic engineering methods. Further, it has been accepted that 'the same physical and biological laws control the behaviour of the organism, whether modified by conventional or r-DNA techniques'. Thus the risk is based on the characteristic of the organism, the modified trait, the environment in which the crop is grown, the interaction between these and the intended application.

While examining the environmental risks from the transgenic plants, it should be recognized that all human activities cause some environmental perturbations. Cultivation of large areas with single species by itself has adverse effect on the environment, even without the use of any fertilizers or pesticides, but can the present human civilization survive without agriculture? There is always a certain amount of risk in all activities which have been accepted in our daily life on the basis of the risks verses the benefits. In the early phase of the r-DNA technologies the 'fear of the unknown' not only in the public but even among the geneticists and molecular biologists led to an extremely cautious approach. However, as the experience with transgenic plants has shown, they do not pose greater risks than the crop cultivars developed using the conventional breeding methods. Yet, as has been pointed out by Deshayes[4] regulations have been implemented to avoid environmental risks. Hence, each crop–transgene combination calls for risk–benefit analysis before initiating the experiments and at all further stages of upscaling.

The major environmental risks from transgenic crops raised and as mentioned by Ghosh are: Weediness of the transgenics (not covered by Ghosh); Gene flow into other cultivars, weedy and wild relatives; Development of new strains of viruses on transgenic virus-resistant plants; Effect of transgenic pesticidal products on non-target organisms; Overcoming the resistance mechanism of the transgenes; Safety of the workers and of food items obtained from transgenic crops.

These have been extensively examined[2,3,5–7] and it would not be appropriate to elaborate them here. Most of the above risks apply to crop varieties developed using conventional methods of breeding and are accepted as a part of the breeding strategy. Though these risks cannot be ignored, it is important to consider the number of independent events that would be required, and the probabilities of their occurrence. For instance, the gene flow into other crop cultivars, wild and weedy relatives, presence of sexually compatible wild and weedy relatives of the crop, flowering phenology of the crop and wild weedy relatives, pollination mechanism, means of pollination, same or different insect species visiting the crop and related species, extent of out-crossing in self-pollinators, pollen tube growth and fertilization after pollen deposition, seed set, viability of seed set under field conditions, germination, growth and fertility of such hybrids and. their natural back crossing with wild and weedy relatives would determine the gene flow. The major concern is for the spread of transgenes conferring resistance to herbicides into wild and weedy relatives which could make them resistant to the commonly used herbicides. Even without growing any of the transgenic crops and with limited use of herbicides in Indian agriculture, development of herbicide resistance in weeds has been observed in wheat fields in Punjab. With the import of semi-dwarf rice and wheat cultivars in the mid-sixties, their extensive cultivation since then has not resulted in any appreciable spread of the semi-dwarfing genes into other cultivars.

Transgenic crops can contribute a great deal towards increasing crop productivity, enhancing the stability of the yield, reducing production costs and use of toxic chemical pesticides and consequently the adverse environmental impact of the production systems. Shelf life of the fruits and vegetables can be enhanced to reduce post-harvest losses. Therefore advanced countries have adopted a more pragmatic approach based on risk–benefit analysis.

India has lagged behind China and other developing countries of Asia in field evaluation of transgenic crops. The national efforts in developing such crop cultivars with desirable agronomic traits would take some more time to reach field evaluation. Meanwhile, as stated by Ghosh, two requests for field evaluation of transgenic materials developed abroad and imported by private sector seed companies have been approved. Many other private companies, national and multinational, wish to bring such materials, which have already been approved abroad, in our country. They however, have apprehensions about timely environmental approvals and protection of the Intellectual Property Rights. For environmental clearances experiences gained in other countries should help us in arriving at our own decisions for upscaling and approvals for commercial cultivation instead for repeating the experiments in our environment to establish the safety of specific crop–transgene combinations. At the same time, the scientists aiming to develop transgenic crops with their own efforts should plan ahead to provide all the biosafety data for their constructs. Further, after the approval of the Genetic Engineering Approval Committee of Ministry of Environment and Forests, the transgenic crop varieties should be treated like any other crop varieties developed by the private seed companies or the publicly funded research organizations by the Ministry of Agriculture and the Central Seed Certification Board. There is also an urgent need to create greater public awareness on the biosafety aspects of transgenics in comparison with the conventionally developed crop cultivars, as some environmental activists can create a scare in the public mind based on imaginary issues.

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Indian science as cultural heritage

The post-independence generation of the scientific community has grown up in, and contributed significantly to, the slightly haphazard and breathless expansion and proliferation, both horizontal and vertical, of scientific and technical institutions in the Government, academic and private sectors. No other ex-colonized country – China excepted – has engaged in such a spree of scientific run-making, by strokes all around the scientific field, not all of which can be characterized as elegant or worthy of emulation in the matches to come. But there is no gainsaying that the batsmen, including the scientific cross-bat wielders, have clocked-up a respectable total on the board.

However, one inevitable casualty of this style of play has been the lamentable loss of awareness, appreciation and significance of the cultural heritage and related fine-structure of our scientific endeavour that has been inherited by the children of the post-independence generation.

The celebrations of the 50th anniversary of our Independence provide an historic opportunity to repair and restore the physical artefacts of this inherited scientific legacy. I have in mind such sites as the scientifically constructed infrastructure of our historic maritime trade and astronomical observatories; such legacies as the laboratory of Ronald Ross in Hyderabad, the great trigonometric survey, the Pasteur Institute, and the artefacts belonging to the Allahabad–Calcutta 'research axis', which axis was closely associated with the freedom movement.

We are, of course, notorious for not having any sense of history at all – and that goes also for several members of the scientific leadership as well. So, the primary purpose of the above exercise would be to establish and convey a sense of connectivity between the present and the efforts, both modest and titanic, of the principal actors of the immediate and more distant past. Without an established and transmitted connection with the artefacts and the actors of the past, many of the present generation, and an alarming number of the next, will view the Indian scientific enterprise as an imported consumable, merely locally import-substituted in hard times, and not even that when money has been more comfortably provided. One of the many adverse consequences of such a happening will be that, although we will be able to identify – in parts even with justifiable pride – science in India, we will be hard-put to identify, map and transmit to the generations to come the continuing story of Indian science.

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Extinct species

Khoshoo (*Curr. Sci.*, 1996, **71**, 506–513) has rightly suggested that India needs a National Biodiversity Conservation Board and it is one of the most welcome suggestions which would help to save our rare species otherwise, they would undoubtedly become extinct if no tangible efforts are made to protect them. However, he has given a sizable list of plants which are alleged to have become extinct in India. Included in this list are two species of the genus *Isoetes* L. (Isoetaceae: Pteridophyta), e.g. *I. dixitei* Shende and *I. sampathkumaranii* Rao, a plant to which we have devoted three decades of pointed attention.

I. sampathkumaranii was initially described by Rao (*Curr. Sci.*, 1944, **13**, 286–287) along water margins in shallow depression in granite rocks from Government Botanical Gardens, Bangalore. This species might have become extinct as its type locality was subsequently destroyed to beautify the garden in question. Moreover, to the best of our knowledge and belief a search for this species at the above type locality and also elsewhere, has yielded nothing positive. There is no later report of its occurrence during the last 25 years or more from any other region.

I. dixitei was for the first time described by Shende (*J. Univ. Bombay*, 1945, **B14**, 50–52) from Panchgani, Maharashtra as occurring in the shallow rock pools on the Tableland. It is specifically mentioned in the above paper that this species became extinct in 1868; but strangely enough this species was described only in 1945. It is therefore, indeed curious, that the species, which was actually described and brought on record as late as 1945 is said to have disappeared in 1868. Even otherwise this species today continues to occur not only in its type locality, Panchgani Tableland but other Tablelands of Panchgani as well as Mahabaleshwar, Maharashtra. However, its protection is essential to save it from the ravages of extinction.

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T. N. Khoshoo replies:

The paper was written with the primary purpose of highlighting an urgent need for a National Biodiversity Conservation Board. The national and international response to such a need was indeed overwhelming. To that extent the paper has achieved its objective.

Among other things, the data about extinction of our biota during the last 100 to 200 years was available only with the World Conservation Monitoring Centre. However, before recording the information from WCMC I wrote the following in the aforementioned paper: 'Though no professional studies have been made on the extinction of biota in India, the following species *appear* to have become extinct'.

Based on Srivastava's information, the WCMC may consider deletion of this particular species from the list of extinct species in India. I also wrote the following in the same paper: 'Although the loss of foregoing (24) species has come to light, there may be many more species which have become extinct. A systematic study has to be initiated by BSI and ZSI involving university system.' Let me hope that credible data on endangerment and/or extinction on Indian biota would be generated in India so that one does not have to depend on outside sources.

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Mode of action of isonicotinic acid hydrazide

M. K. Chattopadhyay

Isonicotinic acid hydrazide (isoniazid), or INH, a front line antitubercular drug, was introduced in clinical practice in 1952. But till now its exact mode of action is not clearly known. Though majority of the *Mycobacterial* species are sensitive to 1 µg/ml or a higher concentration of INH, sensitivity of *M. tuberculosis* lies in the range of 0.02 to 0.06 µg/ml of INH[1]. This extreme susceptibility of the tubercular pathogen to INH is a riddle which is yet to be solved.

A number of papers published in the past few years have emphasized the role of the *katG* gene in INH-toxicity. This gene encodes a bifunctional enzyme with both catalase and peroxidase activities in *M. tuberculosis*. Loss of *KatG* activity due to gene deletion[2] or missense mutations[3] was found to be associated with INH-resistance of the pathogen. It was proposed that catalase-peroxidase might convert INH into a metabolically active form *in vivo* or its action on INH might generate toxic oxygen radicals which are actually responsible for the antibacterial properties of the drug. *In vitro* oxidation of INH by the enzyme catalase-peroxidase was also evidenced[4].

Investigations have been performed from time to time to elucidate the molecular nature of the intracellular target of INH or of its active form. Earlier it was known that the drug inhibited mycolic acid biosynthesis in *M. tuberculosis*[5]. Evidence obtained from further studies suggested that its primary target might be enoyl-acyl carrier protein reductase, encoded by the *inhA* gene of *Mycobacterium* and is believed to play a key role in mycolic acid biosynthesis[6,7].

However the postulation about the mode of action of INH in terms of its ability to inhibit mycolic acid biosynthesis failed to explain why *M. leprae* is far less susceptible to INH compared to *M. tuberculosis*. Involvement of some other gene was evident. INH is also inactive against *Escherichia coli* and *Salmonella typhimurium*. In both these organisms *katG* is a part of a oxidative stress regulon containing a number of genes including *aphC* which encodes the small subunit of alkyl hydroperoxide reductase. They are induced by the *oxyR* gene in response to challenge by H_2O_2. Knock out mutations of *oxyR* in *E. coli* and *S. typhimurium* is known to confer INH-susceptibility in them. In a recent investigation *oxyR* was found to be inactivated by multiple lesions in the wild type strain of *M. tuberculosis* which is INH-sensitive. The gene was present in intact form in *M. leprae*. When *oxyR* and *aphC* from *M. leprae* were inserted into *M. tuberculosis* through cosmid vector, the tolerance of the tubercular pathogen to INH was substantially increased. As a plausible explanation of the association of these two phenomena – viz. mutation in *oxyR* and susceptibility to INH – it has been proposed that the function of *aphC* and other genes induced by *oxyR* is to protect the bacterial cell from the metabolically active form of INH and from the free radicals. Due to mutation in *oxyR*, in the wild type strain the drug cannot be detoxified. A constitutive level of oxidative stress response, present in the wild type strain, scavenge the oxygen radicals produced in course of normal metabolism. But in absence of induction by *oxyR* the oxidative defense mechanism of the cell is unable to quench the extra load of free radicals generated in the presence of INH[8].

It is important to remember that though INH is believed to be converted into an active form by catalase-peroxidase, none of the metabolic products of the drug (isonicotinic acid and 4-pyridylmethanol) identified so far in *M. tuberculosis* has any antibacterial activity[9]. With the evidence available at present, toxicity of INH to the tubercular pathogen appears to be a multifactorial phenomenon. Investigation on some other aspects (e.g. mechanism of uptake of INH by *M. tuberculosis*) may provide further clues.

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On the orchid, *Bulbophyllum crassipes* Hook. f. in the Andaman Islands

Confusion existed upon the identity and nomenclature of this plant since its record by Hook. f. from Penang in 1907. Orchidologists like Holttum, Ridley, Taylor, Ker, Lindley, Seidenfaden and others made a series of attempts to solve it to a considerable extent. Although Seidenfaden and Wood[1] as well as Satishkumar and Manilal[2] accorded this taxon a specific rank, we would like to support the views of Pradhan[3] and Sarkar[4] giving a varietal status, *B. careyanum* (Hook) Spreng. var. *crassipes* (Hook. f.) U. C. Pradhan to the Indian plants. The diversity of the scapes and spikes with the colour variation of the flowers would support this treatment, ideally under *B. careyanum* (Hook) Spreng. var. *crassipes* (Hook. f.) U. C. Pradhan as it differs only by the column feature. It is widely distributed from Sikkim through Assam to northern and northeastern Thailand, with several records from Peninsular Thailand and possibly from Malaysia. The occurrence or distribution of this orchid in Andaman and Nicobar islands has not been mentioned by Vasudeva Rao[5], Lakshminarasimhan and Rao[6] in their enumerations. However, Satishkumar and Manilal[2] have mentioned its distribution in these islands. To the best of our knowledge there are no previous records of its collection at Port Blair except the present ones of South Andaman. It closely resembles *B. cupreum* Lindl, but is quite different from it.

Epiphytes: Pseudobulbs are 6–8 cm distant, faintly 4–5 angled or smooth + 3 cm across. Leaves solitary, fleshy, up to 15 cm long. The scapes holding dense spikes measuring up to + 8 cm long, usually arising from the base, occasionally from the tip of the pseudobulbs. Flowers brownish yellow with maroon spots, remain open for 15–20 days, wilt and fade to dark brown. Growing on *Heritiera littoralis* Dryland, along the littoral belt, in the back mangals where it is inundated only during high tide.

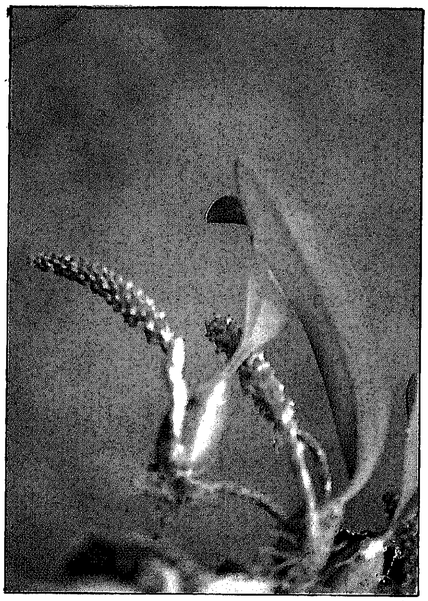

Figure 1. *Bulbophyllum crassipes* Hook. f. from Andaman Islands.

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Does statistical significance always imply biological significance

Innate short-term variation exists in biological data; e.g. daily body weights, dietary nutrient intakes, physiological or biochemical indicators of body status like basal metabolic rates, blood levels of vitamins and minerals and so on. Any physical or physiological stress such as physical training programme, weight control regime or drug therapy alters the mean value of these parameters. To test the significance of the difference between mean values of these parameters, usually Student's t test is used. But application of this test sometimes results in a paradoxical situation. The test declares the difference between means to be highly significant but the experimenter feels that the difference is not worthy of attention. The problem is then whether to call the difference a significant one on statistical grounds or to accept intuition of the experimenter that the difference is negligible. Here we illustrate this issue with examples and describe application of a proper test considering biological variation.

Let $x_1, x_2, \dots x_n$ be the observations on n individuals before treatment and $y_1, y_2, \dots y_n$ after treatment. So $z_i = y_i - x_i$ is the difference between ith pair. Consider the null hypothesis of zero difference; viz. $H_0 : \mu_z = 0$, vs $H_1 : \mu_z \neq 0$ and consider the common situation where the variables are normally distributed. Sample size calculations require the specification of type I and type II errors, a 'difference

Table 1. Effect of physical training on body weight in army cadets

	Before physical training		After physical training		Difference		Paired t test		Interval t	
	Mean	SD	Mean	SD	Mean	SD	t	Decision	δ	Decision
Group 1 ($n = 73$)	56.20	3.19	57.07	3.16	0.868	1.33	5.58	Reject H_0	0.7	Accept H_0
Group 2 ($n = 26$)	55.13	2.73	56.20	3.23	1.070	1.54	3.52	Reject H_0	0.6	Accept H_0

Table 2. Per cent absorption of zinc from two test diets

	Before treatment		After treatment		Difference		Paired t test		Interval t	
	Mean	SD	Mean	SD	Mean	SD	t	Decision	δ	Decision
Diets ($n = 24$)	18.2	2.3	19.8	2.2	1.6	2.4	3.2	Reject H_0	1.2	Accept H_0

to detect' for the means, and the standard deviation (SD) which is usually not known. An overestimate of the SD leads to an overestimate of sample size so that the experiment has more power than expected and can lead to the situation of false significance. Secondly a significant result also can be caused by true difference of means less than those the experimenter wished to demonstrate. The probability of this event will be less than the power previously assumed in sample size calculations, but greater than the type I error. Therefore the 'difference to detect' should not only be included in the planning but also in the test procedure. The issue can be addressed by the following type of hypotheses; $H_0 : -\delta \leq \mu_z \leq \delta$ against $H_1 : \mu_z \leq -\delta,\ \mu_z > \delta$.

These problems have been tackled in statistical research ever since 1938 by Berkson[1], Hodges and Lehman[2] and Anderson and Hauck[3]. In 1993 Mehring[4] has suggested the following procedure as a generalization of Student's t test for testing such an interval hypothesis:

1. Define δ on the basis of practical consideration.
2. Calculate $\gamma_2 = (\delta \sqrt{n}/\sigma)$.
3. Reject H_0, if $1 - F_k(|T| - \gamma_2) + F_k(-|T| - \gamma_2) < \alpha$, (1)

where $T = (z\sqrt{n})/s_z$ is usual t statistic, $s_z^2 = \Sigma(z_i - \overline{z})^2/(n-1)$, and F_k is central t distribution with k d.f., $k = n - 1$.

Table 1 gives the data of body weights of army cadets in training in two phases of activity levels five months apart. Interest was to see the effect of training on body weight[5]. The average difference was 0.87 kg. With the paired t-statistic this difference is significantly larger than zero. This difference, however, was not considered to be a physiologically significant one in view of the duration and regularity in diet as well as exercise. Further, the magnitude of the difference is of the order of day-to-day fluctuations in weight of an individual (between 0.5 and 1 kg)[6]. Logically therefore a difference greater than this value is indicative of a real change in weight. So the test with the value of δ between 0.5 and 1 kg the procedure (1) is carried out. For $\delta \geq 0.7$, and σ known from past data, the null hypothesis that 'the difference between means is not significant' is accepted. Thus the test confirms the findings of Edholm *et al.*[6].

If the value of δ is not previously known, one can estimate it by using within individual variation (Table 2). While testing the difference between per cent absorption of zinc from two diets differing in their compositions, both the diets were given to a group of 24 human volunteers for a period of 15 days each, with a washout period between the two experiments. There was a variation in per cent absorption over days in the same individual. Based upon this within-person variability, value of δ was decided to be the intraindividual standard deviation. The usual t-test showed the difference to be significant. However, incorporating intraindividual variation into the hypothesis to be tested and carrying out the test as explained earlier, it was found that the difference of about 2% in the absorption was not really significant to attribute to difference in the compositions of diets.

Similar problems may arise in medical research where intraindividual variation exists in the parameter under study. Application of the above test with interval $(-\delta, \delta)$ constructed on the basis of intraindividual variation will certainly help in proper analysis and interpretation of the data.

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OPINION

Biotechnology in India: The technology imperative

Harish Padh

The government should de-emphasize basic research and instead provide infrastructure and technology channels for promotion of biotech sector. Facilities like technopark available to software industry if made available to the biotech industry can spur growth of this biotech sector. This will also facilitate proper technology licensing and transfer and promote growth. On a long-term basis, we should also focus on technology development especially for the diseases of the developing countries.

Global prospects for biotechnology industry

In a short span of 15 years, biotechnology has established itself as a significant factor in health-care industry. In USA it has already gained nearly 10% share of the total pharma market. The biotech industry is just coming out of its infancy (Table 1). Its potential is being tested, realized and used. The public awareness and acceptance will accelerate the process. This sector is expected to expand at least 2-fold by the end of the century and will soon match or surpass the computer industry in size, importance and growth. It holds good promise in a number of areas, specially those for which we have no current treatment. There is a tremendous potential for developing countries like India to apply biotechnology in health-care, agriculture and environmental resource management. In the earlier paper[1] I discussed the current status of biotechnology as an industry sector, in this paper I will discuss how India can best benefit from this new set of technologies.

Biotechnology in India

India has a number of good academic institutions with expertise in basic sciences relevant to biotechnology. India also has a fair number of suppliers and stockists who market required reagents and supplies. There is also a surge in testing facilities, R&D institutions, small medical equipment fabricators, repackers and sellers of relevant imported materials.

India has a rapidly swelling population of upper-class and middle-class, needing newer diagnostics and therapeutics. Moreover, proliferating private medical and hospital care has created a tremendous demand for biotech products. Proliferation of diagnostics is mostly met by imports, primarily because there is a general failure of locally developed diagnostics.

Indian pharma industry

Indian pharmaceutical industry is strong and has the expertise for chemical drugs. It is competing well in bulk drug market, largely due to the more efficient nature of the processes and manufacturing costs. The same can hold true for the biological drugs, but at the moment it has little experience in biotech diagnostics and no experience in biotech therapeutics. India's economic liberalization and signing of GATT and Dunkel Draft clear the way and need for significant R&D activities by pharmaceutical industry. Impending changes in patent law will make it necessary to either develop our own technology or obtain proper licence from others. Globalization of economy and liberalization of our economy make this an appropriate time to seek outside licensing and technology transfer. The Western India (Gujarat, Maharashtra), with more than 50% of the registered pharmaceutical units, accounts for 90% of the pharmaceutical production. This region also accounts for more than 70% of import and export of the pharmaceuticals. The Indian pharmaceutical industry will sooner or later enter in manufacturing of biotechnology-based diagnostics and therapeutics. There is no government institution or university in this region with expertise in this area to help the pharma industry.

Table 1. Ten years history of the biotechnology therapeutics in USA

	1985	1990	1995
Sales ($ in billions)	1.1	2.9	9.3
Revenues ($ in billions)	2.2	4.7	12.7
R&D expenditure ($ in billions)	1.7	2.6	7.7
Number of companies	850	1,107	1,308
Employees	40,000	66,000	108,000

Diagnostics

Worldwide there are about 600 new biotechnology-based diagnostics in the market with a value of about $ 20 billions. Many more are about to enter the market, the most prominent among these will be nuclear probe and PCR-based diagnostics. India relies on imports for many of the immunodiagnostics kits. Many of the locally developed diagnostics have failed, while the imported diagnostics are either unsuitable or expensive.

Therapeutics

Expression of foreign genes in convenient prokaryotic cells and the large-scale production of gene products is now routine. These protein products could have applications as therapeutics, diagnostics, restriction enzymes or industrial enzymes. At present there are about 79 biotechnology-derived therapeutics approved for human use in USA. The total market value of these products is about $ 50 billions. About 150 companies have 700 more products in various stages of clinical trials and development. With increasing acceptability of biotech products, there will be about 200 biotechnology-derived therapeutics available in the market by the turn of the century. In 1987 the number of new drugs (IND, Investigational New Drug) produced by biotechnology had overtaken IND produced by conventional means (chemical and antibiotic drugs). This is an indication of the trend that, in future, new therapeutics will be made by cellular factories using recombinant technologies. At present, there is no locally manufactured recombinant therapeutic product available in the market. Few imported biological therapeutic products are marketed in India, e.g. human insulin and streptokinase.

Factors influencing biotech growth

Table 2 shows various factors influencing growth in a particular segment of economy. For example, in USA the growth is driven by innovation while in Germany it is driven by capital investment and proper technology management. In Thailand (and India?) the factors like cheap labour or availability of raw materials, etc. can spur growth. In India and China, sudden growth is evidenced owing to the rapid opening of huge consumer markets. Thus, India is poised to enter the biotechnology market. Infusion of foreign technologies and collaboration has already taken place in other sectors of economy. It is now time for Biotech/Pharma sector to seek proven technologies from outside. The government should provide proper channel and infrastructure to the pharma industry. This will lead to value-added better stable, tested and validated products in the market. Other governments, including Germany has government-supported agencies for technology identification and import. The SIDCs (state industrial development corporations) should get serious about this sector. In addition, the problems of developing countries (malaria, tuberculosis, etc.) have remained untouched by biotechnology either in India or overseas. These problems will need innovative approaches.

Development of new technologies

India has a good number of government institutions and universities involved in the creation and development of new relevant technologies. We have a chain of CSIR institutions and other places like CCMB, IMTECH, NIPER, NII, IISc, etc. engaged in research and development that may help health-care sector. In spite of this modest but consistent support from the central government, we have not utilized our resources and infrastructure to the fullest. I believe primarily it is the academic nature of these institutions which lack appreciation of industry and the market place, resulting in their inability to help industry.

Commercialization of new technologies

There are a few instances where a usable technology has been developed in an academic institution, primarily for diagnostics. However, in most of these cases technologies failed in development stages or even after marketing. This is another example where academia and industry show lack of understanding and appreciation of each other. Our pharma industry is by and large without significant R&D activities because of lower profit margin. Because academia cannot really do development of technologies, and because the industry is either burnt out by academia or unable to appreciate or afford development of technologies, some useful technologies go undeveloped. The net result is that we in India develop far fewer technologies than what our capabilities are and our demands require. Even fewer technologies are actually commercialized. Therefore, we depend on import of products from abroad to meet our growing needs. This hold true for most of the biotech diagnostics and therapeutics.

Academic–industry relationship

In India the academia–industry relationship has worked quite well for chemical industry and other engineering-based industries, primarily because of IITs. As far as biotechnology is concerned, academic–industry relationship is nonexistent or ineffective. By and large each side has a lack of understanding or appreciation of other. At times it translates into outright apprehension (see *Curr. Sci.*, 1991, **60**, 524–528).

Technology absorption and transfer

Since we do not invent and develop technologies, we rely on import of either products or the technologies. Proper licensing and transfer of proven technologies is not that common in India. The process patent policy helped us to 'improvize' processes and it was not needed to go for proper technology licensing. At the same time, very few government institutions worked to facilitate the absorption of technologies. A noted exception is BRIT, a DAE agency which developed indigenous radioimmunoassay kits and made it so user-friendly for Indian conditions that now about 20 diagnostic radioimmunoassay kits are developed and marketed by BRIT. There are about 200 diagnostic centres throughout India where BRIT-trained technicians operate these diagnostics.

Biotech generic market

In the international market, biotechnology drugs are very expensive. For example, Genetech's tPA is priced at about $ 2000 per injection and streptokinase is marketed at about $ 200 per injection. A genetically engineered Factor VIII used in the treatment of hemophiliacs costs $ 25,000 a year. Imported therapeutics traded in India are exhorbitantly priced (Rs 300 per dose of human insulin compared to Rs 65 per dose of traditional insulin) and about Rs 4000 per dose of streptokinase. What will be our strategy when in three years there will be over 200 therapeutics and vaccines available? Shall we still rely on imports? The next generation of these products will have to be less expensive and more effective. We can provide inexpensive manufacturing base for Indian as well as export market. We should go for proper technology transfer and develop the manufacturing base. In addition, many of the biotech products will be off-patent

Table 2. Driving forces influencing industrial growth

Driving force	Precondition	Strategy	Example
Factor	Factor advantage	Low tech sectors	Thailand, India??
Investment	Mature user industry	Investment in getting foreign technology, joint government and private ventures	Germany
Innovation	Developed science base, mature industry base, venture capital	New companies led development	USA
Market base	Sudden opening of large consumer market	Careful import and promotion of proven technologies	India, China, Eastern Europe, Former Soviet countries

in a few years. Priority could be to manufacture these off-patent products. India with proper technology transfer can develop its niche in off-patent biotech 'generic' market.

Need for licensing and transfer of technologies

We have witnessed that indigenously developed diagnostics have shown less than satisfactory performance in the market. The imported diagnostics are not suitable because they are expensive, not against local pathogenic strains, and with little or no quality and stability controls. In addition, the Indian pharmaceutical industry has little or no experience in modern diagnostics or biological therapeutics. Their approach of process improvization that worked well for chemical drugs will not work in biotechnology-based diagnostics or therapeutics. In addition, the number of newer diagnostics in the international market every year is burgeoning. Therefore, the national interest is better served by systematic approach in identifying, licensing and transferring of appropriate proven technologies.

Need for technology development

There are a few diagnostics developed in the country but overall performance has been dismal. There seems to be a gap of culture, communication or something between the academic and corporate worlds as a net result of which the internally developed technologies have not been designed or developed properly. The products do not reach the market or fail in the market. For a long-term interest of the nation there is a need for local development of technologies, especially against conditions like tuberculosis and malaria rampant in India.

What the government can do?

The growth of biotech sector in USA is innovation-based while in other Western countries it is based on technology transfer and capital investment. We ought to realize that every country cannot have innovation-based sector growth (see Table 2). In fact, in biotechnology it is only USA which has witnessed growth of the biotech sector based on innovation. Rest of the countries, including the Western Europe and Japan, have only minor contribution in innovation-based growth. Therefore, it will be futile for India to pour its valuable resources only in research and hope that we too can have innovation-based biotech sector growth. The government can promote biotech sector by the following two approaches. (i) Provide a good infrastructure including a Biotech Park with the state of art common facilities including laboratory and pilot plant equipment. Contrary to belief, these infrastructure facilities can be made financially self-supportive. Benefits in terms of promoting industry are numerous. Many countries including Taiwan, Korea, Japan, European countries, and even Brazil have Biotech Parks to cater to and promote this sector. In India too there are several technoparks to cater to the computer industry. (ii) Establish technology transfer office where entrepreneurs can approach for available proven technology for licensing. This office can shop throughout the world for availability of proven technology, can advise and help entrepreneurs through the negotiation process, and can help industry to get the best deal on import and licencing of proven technologies. At the moment, only the large companies can afford to do their technology shopping on their own. Medium and small scale industry have no such help in identifying and obtaining proven technology. The emphasis is on *proven* technology, one that is successfully used elsewhere. It is a foregone conclusion that technology import or licensing is a must for the near future. It is also evident that small- and medium-scale industry has not been tapped for biotech sector. It has been observed that many entrepreneurs with good investment capacity but who are not trained in biotech would like to diversify in biotech provided they have some reliable technology sources. It has also been observed that many entrepreneurs who were ignorant of technology transfer ended up loosing money in attempts to arrange for the technology transfer on their own. Both these groups of entrepreneurs could be helped by the proposed technology centre.

In summary, the government should de-emphasize basic research and instead provide infrastructure and technology channels for promotion of biotech sector.

Potential concerns for technology transfer

It may be perceived to be an expensive proposition to go for proper technology transfer. However, we do not have any other option. Proper tech transfer adds only a small percentage to the final retail price. Depending on the expected volume, it may cost between 5% and 10% more to the final retail price. Considering the time and expenses saved, this small price is negligible.

Another apprehension is that we will not get a good technology, primarily because of our reputation as technology thieves. In some cases, individual companies have gotten into agreements which were not designed to their advantage. This is mainly due to our inexperience in evaluation of technology potential and negotiation process. And as we enter international business deals, we will have to face and rectify the reputation we might have. Changing our patent laws will be a step in this direction. Another pitfall is that we only look at USA for the technology source. Companies from many other countries have useful technologies while the market in their own countries is limited. These companies are eager to enter markets like India for its local consumers as well as regional export.

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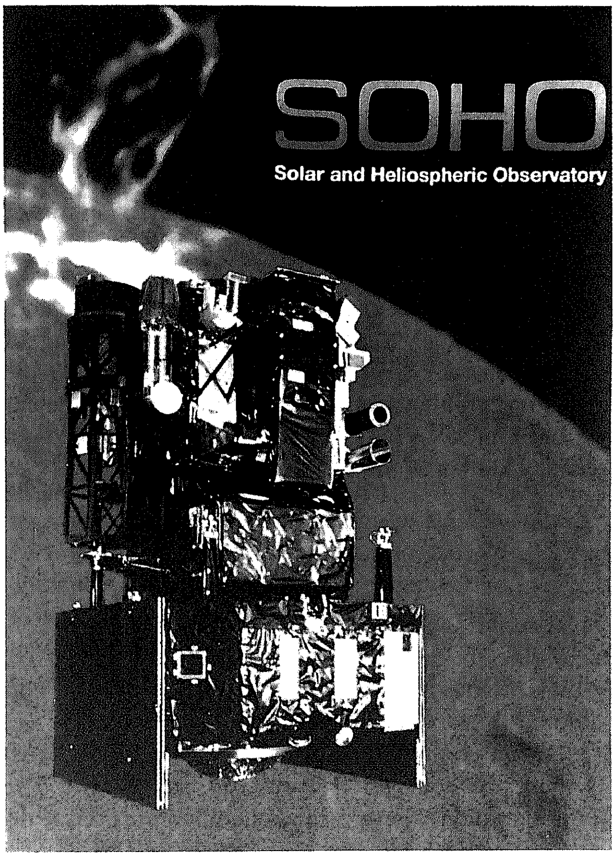
SOHO hunts elusive solar prey

B. N. Dwivedi and Anita Mohan

The Solar and Heliospheric Observatory, SOHO, is a project of international cooperation between ESA and NASA to study the Sun from its deep core to the outer corona and the solar wind. To achieve its goals, SOHO carries a complement of twelve sophisticated, state-of-the-art instruments. In this article, we present a brief account of this ambitious project and highlight some of its achievements since the SOHO spacecraft turned an unblinking eye on a turbulent Sun from its vantage point in space on 14 February 1996.



'All that exists was born from Surya, the God of gods'

Rig Veda

LAUNCHED on 2 December 1995, the solar space observatory SOHO stationed itself into a 'halo orbit' around the L1 Lagrangian point (the point 1.5 million kilometers away from us at which the gravitational pull of the Earth balances that of the Sun) on 14 February 1996 (cf., Figure 1). Since then, the scrutiny of the Sun has gone on 24 hours a day, 7 days a week and will continue for another couple of years or so. SOHO was also a medieval Anglo-French hunting cry; but this time the hunt is for answers to basic questions about the Sun – from its deep core to the outer corona and the solar wind. All previous solar observations were periodically interrupted as our planet 'eclipsed' the Sun. SOHO, a most ambitious project of ESA and NASA, provides the first long, clean uninterrupted view of the Sun to answer some riddles like (i) what are the structure and dynamics of the solar interior?, (ii) why does the corona (the tenuous outer solar atmosphere that can be seen with the naked eye during eclipses and which is much hotter than the solar surface) exist and how is it heated?, and

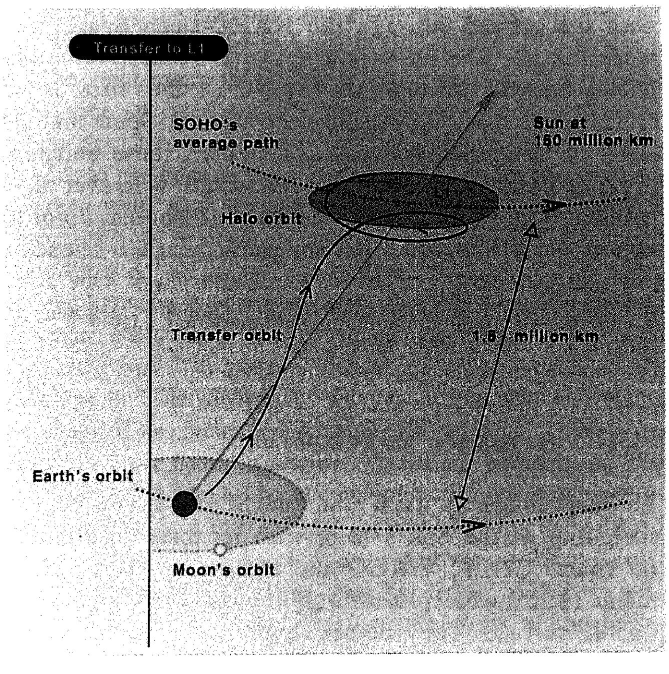


Figure 1. To the sunward station. After its launch on 2 December 1995 by an Atlas II A Centaur into a transfer trajectory to the first Lagrange point L1, SOHO arrived at its vantage point on 14 February 1996 in an orbit 1.5 million kilometers nearer to the Sun than the Earth's own orbit. At L1, or Lagrange point No. 1, the gravitational pull of the Sun is balanced by the pull of the Earth and the centrifugal force acting on a spacecraft orbiting in step with the Earth. SOHO remains hovering around that relative position, in a 'halo orbit', while it accompanies the Earth in its annual march around the Sun.

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(iii) where and how is the solar wind (the particle streams which represent the solar mass loss) accelerated? Among other tricks of the Sun (laboratory for astrophysics), for instance, it is one of the few places where observations and theoretical considerations may give us sufficient insight into a deeper understanding of the effects of magnetic field. This includes its fascinating attribute that, like a biological form, it can reproduce itself. The complexities of magnetic field are fascinating: in some cases it serves to refrigerate the gas (e.g. sunspots) while in others it serves to heat the gas to millions of degrees (e.g. active corona).

SOHO carries a complement of twelve sophisticated, state-of-the-art instruments: Global Oscillations at Low Frequency (GOLF), Variability of solar Irradiance and Gravity Oscillations (VIRGO), Solar Oscillations Investigation/Michelson Doppler Imager (SOI/MDI), Solar Ultraviolet Measurements of Emitted Radiation (SUMER), Coronal Diagnostic Spectrometer (CDS), Extreme-ultraviolet Imaging Telescope (EIT), Ultraviolet Coronagraph Spectrometer (UVCS), Large Angle Spectroscopic Coronagraph (LASCO), Solar Wind Anisotropies (SWAN), Charge, Element and Isotope Analysis System (CELIAS), Comprehensive Suprathermal and Energetic Particle analyser (COSTEP) and Energetic and Relativistic Nuclei and Electron experiment (ERNE). For a detailed description of all these instruments, see ref. 1. The spacecraft maintains contact with the ground through the NASA Deep Space Network (DSN). The DSN is a network of three radio antennas spread around the world – the first is in Goldstone, USA; the second near Madrid, Spain and the third in Canberra, Australia. Together, these antennas provide continuous links to spacecraft wherever they happen to be in relation to the Earth. Planning, co-ordination and operation of the spacecraft and the scientific payload is conducted from the Experiment Operation Facility (EOF) at NASA Goddard Space Flight Center (GSFC).

SOHO probes the Sun's interior

The Sun sings to itself and the SOHO makes a hi-fi recording of the Sun's song with its three instruments on board: GOLF, VIRGO and SOI/MDI. These instruments record widespread throbbing motions of the Sun's visible surface. They detect rhythmic variations in the intensity of light or in its wavelength. The oscillations are caused by sound waves reverberating through the Sun. Just as seismology reveals the Earth's interior by the study of earthquakes, or seismic waves, so helioseismology looks below the Sun's surface. SOHO's helioseismology experiments, conducted from a steady platform in space, provide greater clarity than the ground-based stations (e. g. GONG).

SOI/MDI observes a million points on the Sun's visible surface once a minute. It can detect subtle, short-range oscillations due to sound waves penetrating only a short distance into the Sun. And it has generated the first chart of horizontal motions of gases just below the visible surface, indicating shallow flows. Ground-based instruments have detected motions deep inside the Sun. That can be done with SOHO too, but it also provides the missing link to motions at the visible surface. With the help of first movies of the Sun's interior and by relating them to the measurements of surface magnetic fields, we may begin to solve the mystery of dark blemishes on the Sun: why do sunspots occur and why do they become most numerous every eleven years or so?

The instrument VIRGO monitors large-scale oscillations that penetrate all the way to the centre of the Sun. It shows that the variation of the sound-speed with depth is smoother than in the theoretical models[2]. Theoretical prediction is that the speed of sound should show jumps corresponding to layers of different compositions deep in the Sun. This suggests that the distribution of helium abundance is smoother than in the theoretical models. A smoother distribution of helium would have a profound effect on models of nuclear fusion in the core of the Sun. And such a change in the understanding of the Sun's internal engine could help solve one of the most nagging problems in solar physics: why do detectors on Earth pick up fewer neutrinos than predicted – a discrepancy known as the solar neutrino problem.

SOHO tunes in the Sun's atmosphere

The solar atmosphere is studied by five SOHO instruments. Three of them, viz. SUMER, CDS and EIT, study the chromosphere and the transition region into the corona. Two other instruments, viz. UVCS and LASCO, examine the middle corona between 1.3 and 10 and 1.1 and 30 solar radii from the Sun-centre. The imaging instruments have already yielded surprises, including spectacular solar images that are suggestive of a turbulent Sun. Even though the Sun is currently in the quietest phase of its eleven-year cycle of activity, members of the SOHO team have recorded vigorous action going on in the Sun every day. Short, hair-like jets of strong emissions decorate the solar atmosphere, not clearly seen before. Recorded by EIT, these spicules of various kinds tell of energetic upheavals that may be responsible for heating the outer atmosphere to more than a million degree temperature Celsius. Also visible in the ultraviolet images are plumes-like ropes, stretching far into space from the north and south poles of the Sun. Figure 2 shows the image of the Sun taken in extreme-ultraviolet light at 195 Å from Fe XII ion by the EIT on 9 May 1996. This figure shows solar plasmas at 2 million degrees, confined by magnetic structures. Movies

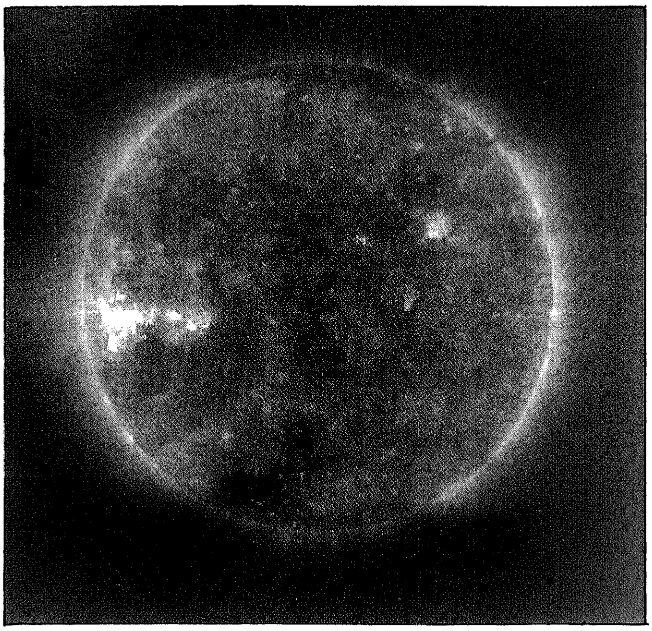

Figure 2. The image of the Sun in the extreme-ultraviolet light at 195 Å from Fe XII ion taken by the EIT on 9 May 1996. This image shows solar plasmas at 2 million degrees, confined by magnetic structures (courtesy, EIT/SOHO team).

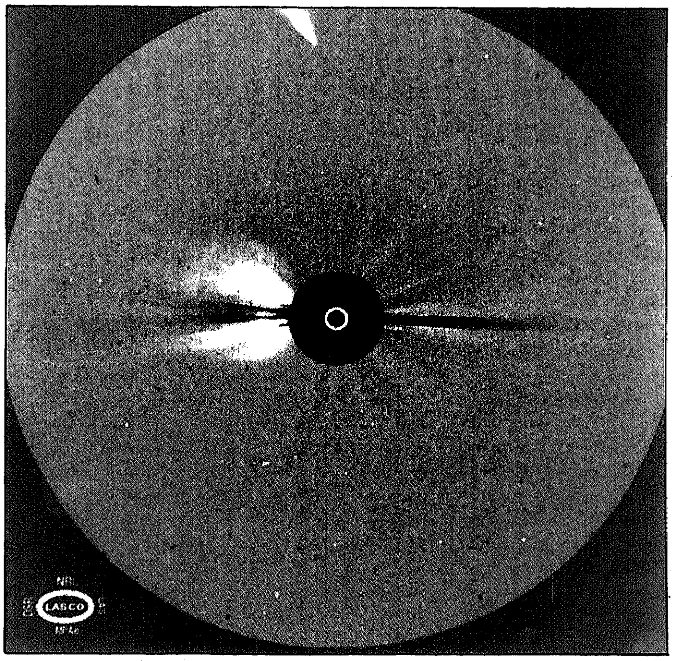

Figure 3. This image was taken using the LASCO on 1 May 1996. The white circle shows the location of the Sun, hidden behind an occulting disc, to allow observation of the diffuse outer layers of the Sun's atmosphere. The image shows an enormous eruption, known as a coronal mass ejection (CME), emanating from the left hand edge of the Sun and travelling out at a speed of several hundred kilometers per second. Also captured on the image (at the top) is the comet Hyakutake on its approach to the Sun (courtesy, LASCO/SOHO team).

of such images have revealed that the solar atmosphere is extremely dynamic, even though it is currently in its 'minimum' state.

Images obtained with SOHO's visible-light coronagraph, LASCO, show the Sun releasing billions of tons of gas into the solar system that can affect the Earth's own space environment. Although the Sun is relatively very quiet, a couple of outbursts have already been recorded by LASCO. Repeated observations over several hours, made from SOHO's vantage point in space where the Sun never sets, results in impressive movies of the events. The coronagraph masks the glaring light from the Sun's visible surface to make the corona observable. LASCO has a particularly wide field of view, out to fifteen times the solar diameter on either side. As it is a composite instrument, LASCO can also observe the atmosphere quite close to the solar surface. This capability of LASCO is helping the interpretation of the mechanism of the coronal mass ejections. For the first time, we see the Sun preparing itself for a mass ejection. In the days preceding such an event, multiple magnetic loops appear in the images of the inner corona. They tell us that the Sun is reorganizing its magnetic field. This destabilizes the solar atmosphere and causes the mass ejection. This observation would enable us to work in advance solar outbursts, endangering low-flying satellites, and may harm power distribution systems on the Earth. Figure 3 shows the LASCO image with an enormous eruption on 1 May 1996. Also captured on the image (at the top) is the comet Hyakutake on its approach to the Sun. Tracking comets by a solar spacecraft reminds us of the fact that the solar wind was discovered by the study of comet tails.

The instrument SUMER is equipped with a normal incidence spectrometer to study plasma flows, temperatures, densities and wave motions from the chromosphere to the corona from ten thousand to over a million degrees with high spatial (1.5 arcsec, 1 arcsec = 725 km) and high temporal (~ 10 sec) resolution. Earlier it was not possible to have a consistent view of the solar atmosphere by measuring line profiles and intensities, Doppler shifts and line broadenings with such a high accuracy from the chromosphere to corona by a single instrument. The SUMER is providing an enormous amount of excellent high-resolution EUV data to address such basic questions in solar physics as the existence of corona and the acceleration of solar wind. Image of the Sun in the EUV light (C IV line at 1548 Å) taken by the SUMER (cf. Figure 4) reveals the million-degree corona. The first results from SUMER will shortly appear in *Solar Physics*[3]. Analysis of the high-quality EUV data from SUMER seem to pinpoint processes that maintain the corona and accelerate the solar wind[4]. Plasma diagnostics applicable to CDS and SUMER instruments have been discussed earlier[5] and hence will not be repeated here. The early indications of SOHO's performance amply justify the creation of a Sun-grazing spacecraft capable of observing ultraviolet

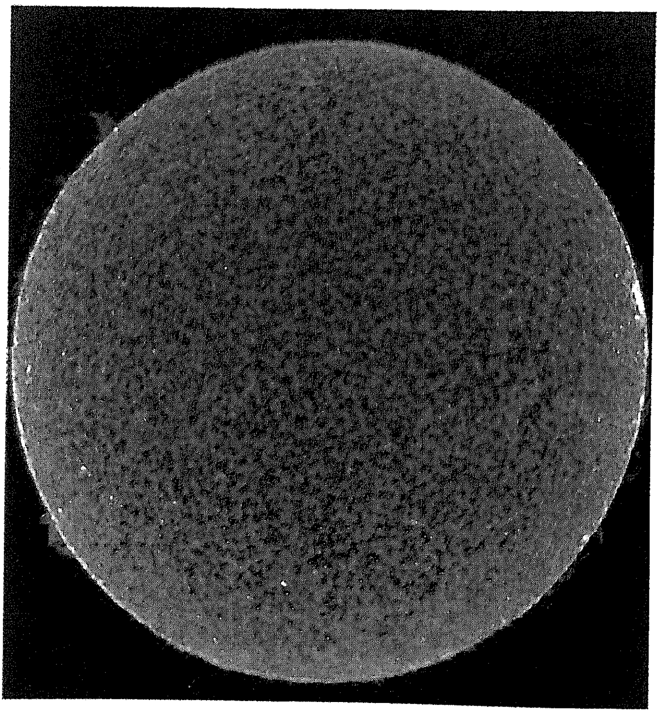

Figure 4. The Sun in the EUV light (C IV line at 1548 Å) reveals the million-degree-temperature corona (courtesy, SUMER/SOHO team).

emissions that are blotted out by the Earth's atmosphere. The imager EIT, ultraviolet spectrometers SUMER and CDS, and the ultraviolet coronagraph UVCS (an imager for the outer atmosphere) are being used to analyse the violent processes on the Sun at a wide range of wavelengths. Interpretation of these observations is likely to give us a much better insight of the Sun.

SOHO probes the solar wind *in situ*

The hot solar atmosphere is expanding into interplanetary space, filling the solar system with a perpetual flow of electrified matter called the solar wind. Spacecraft have made *in situ* measurements of the solar wind near the Earth, showing that it manifests itself in two ways, either as wind moving at a relatively slow speed of 300 to 400 km/s, or as high-speed streams of 600 to 800 km/s. What forces propel the solar wind to these supersonic speed with such tremendous energy, and where do the components of the solar wind come from? This is one of the principal objects of the SOHO mission through its three instruments on board, CELIAS, COSTEP, and ERNE. By analysing the solar wind, SOHO investigators have learnt that the relative abundances of different isotopes of magnesium in the solar wind are close to those found on Earth. This indicates that the Sun's cool surface probably preserves a good sample of the primordial solar system's makeup.

SWAN instrument on SOHO surveys the sky around the Sun to examine the ultraviolet glow from hydrogen atoms lit by the Sun. These atoms come on a breeze from the stars that blows through the solar system. But the competing wind of charged particles from the Sun breaks the incoming atoms, so that they no longer emit their characteristic wavelength. The result is a hole in the pattern of emissions downstream from the Sun. The surviving emissions are brightest upstream, and far above the plane of the Sun's equator. Accordingly, it appears that the solar wind blowing from high-latitude regions of the Sun is less strong than from lower latitudes, at least during the present quiet phase of the eleven-year cycle of solar activity. The Earth is also visible in the maps being produced by SWAN because a cloud of hydrogen gas called the 'geocorona' envelops it and glows in the ultraviolet light. The 'geocorona' would hamper observations of the interstellar glow by satellites close to the Earth. As SOHO sees the geocorona from the outside, it can monitor the effects of solar activity on the Earth's outer atmosphere. At the present time of a quiet Sun, the sky maps clearly indicate a situation of increased solar wind around the Sun's equator. What will happen when the Sun becomes stormier remains to be seen. This will then enable to study important changes in the solar wind's impact on the interstellar gas, revealed by the changes in the sky maps.

SOHO makes sense of the moody Sun

Observing the Sun in different ways, SOHO will link events in the Sun's atmosphere and solar wind to activity beneath the visible surface and the Sun's strange song – to understand the Sun better, and to learn how it rules the interplanetary space and the Earth's environment. In the next two years or so, SOHO will unravel some of the mysteries of the Sun. And William Butler Yeats says, 'I will . . . pluck till time and times are done . . . the golden apples of the Sun', the enthralled SOHO scientists have only just started.

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Effect of greenhouse gases on climate change and Indian ruminant livestock

G. P. Singh

All countries are concerned about environmental pollution causing depletion of the ozone layer. Global warming is an international problem. But health hazards due to toxic gases in air differ from country to country and also from city to city, depending on their industrial status. This article discusses the emission of greenhouse gases and the role of Indian ruminants in respect of methane production.

Methane production by ruminant livestock

METHANE emission in atmosphere as greenhouse gas is estimated to be 255 (ref. 1), 423 (ref. 2) and 550 (ref. 3) million tonnes per year from all sources. According to the World Resource Institute (WRI) estimate, Indian contribution from all sources is 12.1% of the total production. Rice field and ruminants contribute 27.3% and 13.2%, respectively (Table 1). Therefore, measures may be taken to reduce methane production from rice field and ruminants. According to Bolls *et al.*[4], global methane production comes from non-biological material (32%), rice field (18%), ruminants (18%), marshes (13%) and other sources (6%).

Reaction of methane in atmosphere

The most important reaction of methane in atmosphere is the conversion of ozone (O_3) to O_2 and O.

$$OH + CH_4 \rightarrow H_2O + CH_3$$

$$O_3 + h\nu \rightarrow O + O_2$$

$$O + H_2O \rightarrow OH + OH.$$

The reaction of methane in atmosphere is a complex one as: (i) Methane has absorption band in infrared region. (ii) It is oxidized in troposphere by free radical OH. (iii) It is a sizeable source of CO through its oxidation by OH. (iv) It is a source of water vapour in the stratosphere owing to its oxidation to CO_2 and H_2O. (v) Stratospheric methane can react with Cl radicals, forming HCl which slow the rate at which Cl and ClO destroy stratospheric ozone.

Methane plays different roles under different spheres of O_3.

Stratospheric O_3

$$O_2 + h\nu \rightarrow O^*$$

$$O^* + O_2 \rightarrow O_3$$

$$CFC \rightarrow Cl$$

$$Cl + O_3 \rightarrow ClO + O_2.$$

Tropospheric O_3

$$O_2 + h\nu \rightarrow O^*$$

$$H_2O + O^* \rightarrow OH + OH.$$

Methane

$$OH + CH_4 \rightarrow CH_3O + H_2$$

$$NO_2 + h\nu \rightarrow NO + O$$

$$O + O_2 \rightarrow O_3$$

$$NO + O_3 \rightarrow NO_2 + O.$$

Looking into stoichiometry of hexose fermentation, it is clear that:

$$C_6H_{12}O_6 \rightarrow 2CH_3COOH + 2CO_2 + 8[H]$$
(Methanogenic)

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2COOH + 2H_2O + 4[H]$$
(Glucogenic)

Table 1. Total methane emission in atmosphere and methane emitted in India by different sources (million tonnes)

Source	World	India	Contribution of India as of world
Solid waste	44.0	1.80	4.1
Livestock	76.0	10.00	13.2
Hard coal	16.0	0.83	5.2
Rice field	66.0	18.00	27.3
Pipeline leakage	53.0	0.18	0.3
Total	155.0	30.80	12.1

Based on WRI 1990 data.

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$C_6H_{12}O_6 \rightarrow CH_3CH_2CH_2COOH + 2CO_2 + 4[H]$
(Methanogenic).

Twelve [H] are produced; however, 4[H] are utilized for propionate formation and 8[H] are converted to methane.

$$4H_2 + O_2 \rightarrow CH_4 + 2H_2O.$$

This is a theoretical calculation and may vary from feed to feed. The molar proportion of volatile fatty acids (VFA) plays a key role because fibrous feed results in higher methanogenic VFA[5]. Formate, methanol, methylamine, dimethylamine, acetate, etc. are also formed in the rumen owing to primary fermentation of carbohydrate, which are converted to methane to some extent by methanogens. Therefore, methane production control is possible at the rumen level by manipulation of the diet, or managemental practices and by modifying the microbial activity in rumen.

Supplementation of nutrients to straw-based diet

In India and in most of the developing countries, cereal straws are the major feed for ruminants. These are poor sources of nitrogen and minerals and energy, because of being present in the form of cellulose/hemicellulose, which on fermentation give rise to higher methanogenic VFA, and produce more methane[6]. Supplementation of straw diet with deficient nutrients however optimizes the rumen fermentation leading to reduced methane and carbon dioxide production[7] and increases the efficiency of growth and milk production.

Supplementation of UMM lick to crop residue diet

Percentage of methane in greenhouse gas decreased from 41.3 to 38.2 in untreated wheat straw and from 38.6 to 35.4 in urea-treated wheat straw owing to supplementation of urea–molasses–mineral (UMM) lick. Thus methane in greenhouse gas reduced by 5.8% to 7.5% and 6.0% to 8.0% on untreated and urea-treated straw, respectively. However, the percentage reduction of methane in greenhouse gas was 12–15 due to both supplementation of UMM lick and urea treatment[8]. Molar percentage of acetate decreased significantly due to supplementation of UMM lick to straw-based diet. The percentage increase of molar propionate decreased the methanogenic VFA[5]. Changes in molar proportion of acetate, propionate and butyrate due to supplementation of UMM lick are described as the shift in the fermentation process[9].

Percentage of methane in gas decreased from 42.2 to 36.5 due to supplementation of UMM lick to paddy straw-based diet, resulting in about 9% decrease of methane in greenhouse gas on untreated paddy straw, and from 39.7 to 35.5% on urea-treated paddy straw, the reduction being to the extent of 11%. Methane production decreased from 2.76 moles/kg digestible dry matter (DDM) to 2.30 moles/kg DDM due to supplementation of UMM lick and urea treatment of paddy straw, resulting in 16.7% reduction of methane[8]. Increase in propionate due to supplementation affects the methane in gas (Figure 1) because propionate acts as a hydrogen sink[8,10].

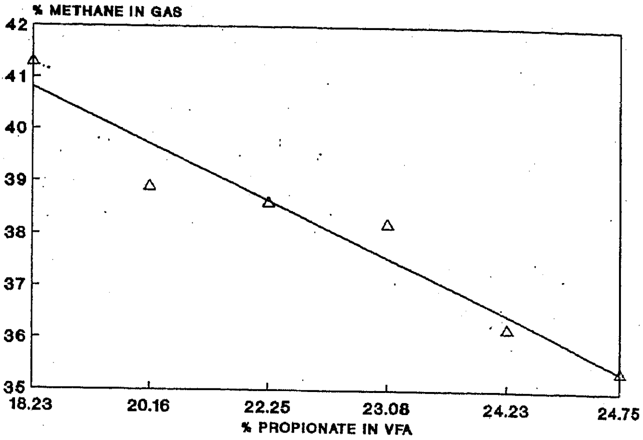

Figure 1. Relation between percentage of propionate in volatile fatty acids and percentage of methane in gas.

Supplementation of urea, mineral and by-pass protein

For the ruminants fed mainly on straw, the methane generated per kg meat is estimated about 2 kg methane per kg meat produced. However, by supplementing with urea, minerals and by-pass protein, methane production is 0.36 kg per kg meat due to increased efficiency of fermentation, digestion and faster growth[11].

Concentrate roughage ratio in ruminant diet and methane production

Sicilliano-Jones and Murphy[12] reported significant decrease in acetate and increase in propionate when steers diet was changed from alfalfa hay : concentrate feed of 4 : 1 to 1 : 4. On the other hand, Davies[13] reported 67%, 21% and 12% acetate, propionate and butyrate in VFA, respectively on alfalfa : grain (1 : 1.3) diet and 49%, 40% and 11% acetate, propionate and butyrate on alfalfa : grain (1 : 6.6) diet. Beever *et al.*[14] calculated the VFA and methane production on different types of diets:

(i) *High forage diet*

1 mol CHO $\rightarrow$ 1.34 mol CH_3COOH + 0.45 mol

$$\mathrm{CH_3CH_2COOH + 0.11\ mol\ CH_3CH_2CH_2COOH + 0.61\ mol\ CH_4 + 4.62\ mol\ ATP}.$$

(ii) *High cereal diet*

$$\mathrm{1\ mol\ CHO \rightarrow 0.90\ mol\ CH_3COOH + 0.70\ mol\ CH_3CH_2COOH + 0.20\ mol\ CH_3CH_2CH_2COOH + 0.38\ mol\ CH_4 + 4.38\ mol\ ATP}.$$

(iii) *High molasses diet*

$$\mathrm{1\ mol\ CHO \rightarrow 0.94\ mol\ CH_3COOH + 0.40\ mol\ CH_3CH_2COOH + 0.33\ mol\ CH_3CH_2CH_2COOH + 0.54\ mol\ CH_4 + 4.50\ mol\ ATP}.$$

However, the above results based on good quality roughage like alfalfa and grain-based feed are not applicable for Indian ruminants because their major roughage is straw and the concentrate mixture contains agro-industrial by-product.

One of the important methods of reducing the methane production is by increasing the concentrate mixture in diet, specially when animals are fed straw-based diet. Singh and Mohini[15] reported that propionate in VFA increased from 23.33% on concentrate-25 : wheat straw-75 diet to 35.60% on concentrate-75 : wheat straw-25 and 30 on concentrate-50 : wheat straw-50. Methane production reduced from 40.9 to 28.0 lit/kg DDM due to the increase of concentrate mixture from 25% to 75% in wheat straw-based diet (Figure 2). Similar studies on three ratios of concentrate and paddy straw (25 : 75, 50 : 50 and 75 : 25) showed an increased propionate percentage in VFA acids (22.3%, 29.5% and 36.7%) and decrease in methanogenic VFA from 77.7% on concentrate-25 : paddy straw-75 to 70.5% on concentrate-50 : paddy straw-50 and 63.3% on concentrate-75 : paddy straw-25, respectively[16]. Thus, methane production was reduced by 19.5% to 31.5% due to increase of concentrate in wheat-straw-based feed. However, the reduction of methane production was between 20.4% and 26.6% when diet was on straw alone and when concentrate mixture was increased up to 75% in the feed based on paddy straw as roughage (Figure 3).

Green fodder in diet and methane production

The green fodder and grasses contain higher amounts of soluble sugars, which when fed to ruminants change their fermentation pattern towards more propionate and less methane production. *In vitro* methane production studies on different ratios of berseem and wheat/paddy straw resulted in 20–30% and 18.5–30% reduction in methane production depending on the ratio of berseem

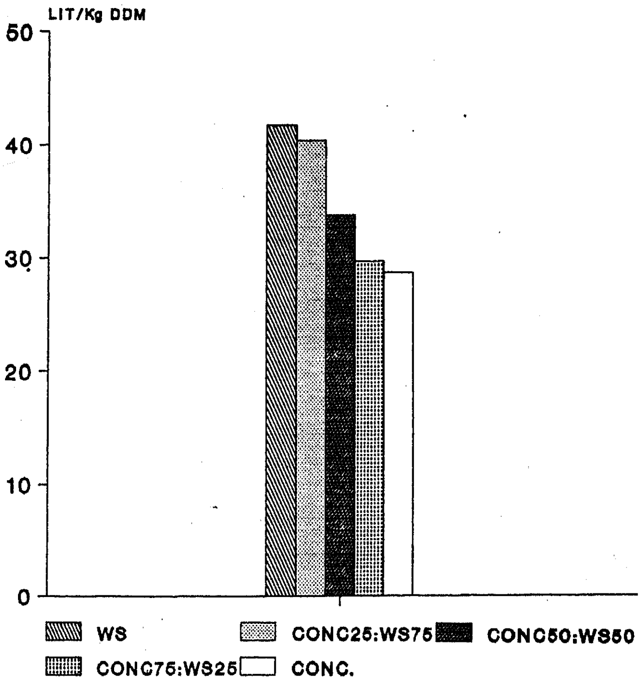

Figure 2. Effect of concentrate and straw ratio in diet on methane production.

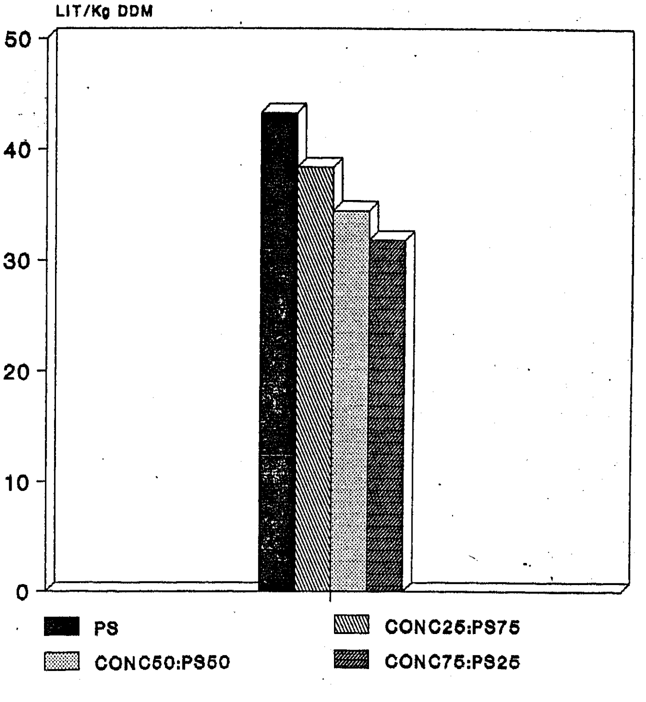

Figure 3. Effect of concentrate and paddy straw ratio on methane production.

Table 2. Effect of rumensin feeding to ruminant on the molar percentage of acetate, propionate and butyrate in volatile fatty acids

	Rumensin fed (mg/day/animal)		
	0.0	50.0	100.0
TVFA (mg/100 ml SRL)	11.16 ± 0.63	12.01 ± 0.48	11.52 ± 0.78
Molar percent of			
Acetate*	65.85 ± 1.32^{a}	58.81 ± 1.05^{b}	58.86 ± 0.27^{b}
Propionate**	24.33 ± 0.99^{a}	32.73 ± 1.81^{b}	32.29 ± 0.58^{b}
Butyrate	9.79 ± 0.86	8.46 ± 0.30	8.85 ± 0.58
Methanogenic VFA*	75.64 ± 1.86^{a}	67.27 ± 2.10^{b}	67.71 ± 2.37^{b}

Figures with superscripts in a row differ significantly.
*$P < 0.05$.
**$P < 0.01$.

in diet. In oat green fodder and wheat straw combinations, methane production reduced to the extent of 8–23%. Therefore, in India, methane production could be reduced to a significant extent by feeding green fodder to ruminants and thereby manipulating rumen fermentation.

Feed additive for minimizing methane production by ruminants

A large number of inorganic, organic compounds and ionophores are known to modify microbial activity to reduce methane production, for example as carbon tetrachloride, which is a very effective, methane inhibitor but toxic to animals and thus cannot be used for feeding[17,18]. The most promising and tested rumen fermentation modifier is momensin/rumensin. Ionophores are routinely used as feed additive in beef cattle feed in the US. Ionophores have little direct effect on methanogens, but they inhibit the growth of bacteria which produce hydrogen, the precursor of methane[19]. Rumensin/momensin increases the feed utilization efficiency and this effect can be mediated by a decrease in methane production, with increase in propionate and ruminal pH (ref. 20). In a rusitec experiment, it has been reported that feeding of 0, 2, 10 and 50 mg/day momensin resulted in 48, 30, 19 and 18 mol/day methane production, indicating the effectiveness of momensin for control of methanogenesis[21,22].

It has been observed that the feeding of 50 and 100 mg rumensin/day/animal increased the propionate production significantly and decreased the percentage of methane in gas as well as methane produced per unit digestible dry matter (Tables 2 and 3). The methane production was reduced by 14–23% on maintenance, 30–35% on medium milk production and 22–32% on high milk production feed consisting of concentrate and wheat straw due to rumensin feeding[23]. Similarly on three types of feed consisting of concentrate and paddy straw, propionate production increased and methane produced per unit of digestible dry matter decreased

Table 3. Gas and methane production on concentrate and wheat/paddy straw diet as affected by rumensin

Rumensin (mg/d)	Total gas (lit/kg DDM)	Methane in gas (%)	Methane production (lit/kg DDM)
Wheat straw*			
0	108.8	38.8	39.99
50	100.9	31.9	32.23
100	98.9	31.0	39.39
Concentrate*			
0	111.7	25.6	28.71
50	111.4	17.1	18.93
100	125.6	13.2	16.62
Concentrate-25 : wheat straw-75*			
0	114.1	33.2	41.48
50	124.1	28.5	35.41
100	122.1	26.1	31.83
Concentrate-50 : wheat straw-50*			
0	110.5	30.6	33.83
50	110.5	21.4	23.68
100	117.9	18.5	21.81
Concentrate-75 : wheat straw-25*			
0	108.7	26.5	28.71
50	107.2	22.5	22.25
100	105.1	18.7	19.67
Paddy straw**			
0	111.3	37.3	38.59
50	108.6	33.0	33.16
100	107.3	32.2	30.66
Concentrate-25 : paddy straw-75**			
0	109.7	34.1	42.08
50	109.3	28.8	35.84
100	111.2	25.2	32.77
Concentrate-50 : paddy straw-50**			
0	112.3	31.0	33.63
50	111.5	22.8	25.86
100	107.3	20.1	22.28
Concentrate-75 : paddy straw-25**			
0	108.7	27.3	27.50
50	108.1	23.2	23.68
100	110.3	18.1	20.55

DDM = Digestible dry matter.
*Singh and Mohini[23].
**Singh and Mohini[24].

(Table 3). Reduction in methane production was 15–22% on maintenance production, 23–32% on medium production and 14–25% on high milk production diet due to feeding of 50 and 100 mg rumensin per day[24]. On the basis of digestibility and methane production per kg DDM methane production by 400 kg cow was calculated at maintenance, medium and high production feeds and it was observed that the methane production was reduced from 153.1–162.0 to 112.8–129.0 lit/day at maintenance level of feeding (concentrate-25 : straw-75), from 178.0–183.3 to 119.0–127.9 lit/day at medium level of production ration feeding (concentrate-50 : straw-50) and from 202.8–206.8 to 146.3–157.60 lit/day on high production ration (concentrate-75 : straw-25) feeding due to rumensin in the diet (Figure 4). Thus, Table 4 shows that the methane production by Indian ruminants is lower than the reported values[1,17] and the measures suggested above could reduce methane production by 20–30% or 2–3 million tonnes less per year by Indian ruminants than the WRI[1] estimate of 10 million tonnes methane per year. Therefore, Indian livestock contribution to greenhouse pool could be brought to 9.2–10.5% from 13.2% of the total methane produced by animals all over the world. This will increase the energy utilization efficiency of animals and decrease the methane level in greenhouse gases pool which is responsible for gradual depletion of ozone layer and global warming.

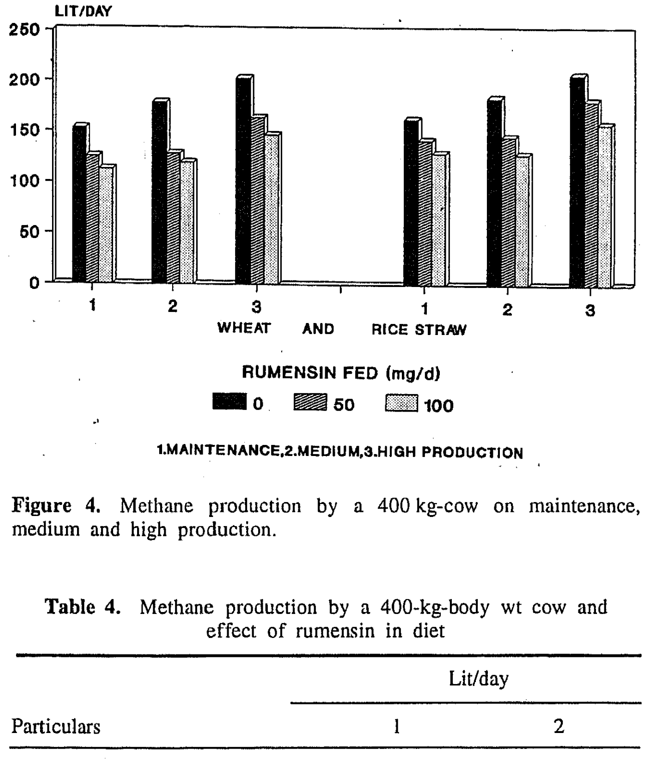

Figure 4. Methane production by a 400 kg-cow on maintenance, medium and high production.

Table 4. Methane production by a 400-kg-body wt cow and effect of rumensin in diet

Particulars	Lit/day 1	 2
Maintenance	154.0	150–270
Medium milk production (5–10 mg/day)	175.0	–
High milk production (10–18 kg/day)	202.0	280–300
Rumensin in diet		
Maintenance	125.0	–
Medium production	127.0	–
High production	163.0	–
Sheep	14.18*	26–36

1. Singh and Mohini[16,23,24].
2. Czerkawski[17].
*Singh and Leng[28].

Total methane emission by Indian ruminants

In rural areas of India, animals are poorly fed. They are fed on crop-residue-based feed and whatever is available at the farmer's house. Feeding practices and type of feed differs from region to region. Even the body weight of animals is not same and varies to a great extent. Keeping all these factors in mind, total emission of methane from ruminant livestock has been calculated based on the 1993 level of animal population (Table 5). Total emission of methane by cattle is 5.690 Tg/year. While males contribute 3.088 Tg/year, females only contribute 2.602 Tg/year. Buffaloes contribution of methane to atmosphere is estimated to be 2.735 Tg/year[25]. Male buffalo emits only about 18% of methane and the rest is by female buffaloes. Sheep and goat contribution of methane in total methane pool was

Table 5. Methane production by Indian ruminant livestock (cattle, buffalo, sheep and goat)

		Methane production		
Species	Number (× 10^3)	Lit/day (× 10^8)	Mole/day (× 10^8)	Tg/year
Cattle crossbred				
Male	4278	5.00	0.26	0.154
Female	8513	12.48	0.56	0.322
Cattle indigenous				
Male	94407	112.60	5.00	2.934
Female	88512	87.50	3.90	2.280
Total	195867	218.50	9.72	5.690
Buffalo				
Male	16706	19.10	0.85	0.498
Female	60054	85.80	3.83	2.237
Total	76760	108.90	4.86	2.735
Sheep	44837	8.00	0.36	0.388
Goat	99405	14.90	0.66	0.388
Total				9.023

Based on:
1. Animal No.: Tech. Committee Report, Govt. of India, 1993.
2. Body weight of animal.
3. Type of feed and intake.
4. Digestibility of feed.
5. Methane per kg DDM determined.

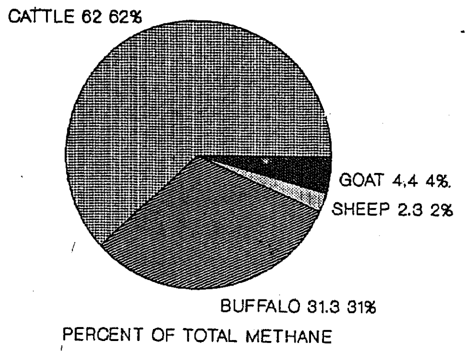

Figure 5. Contribution of methane by different ruminant livestocks.

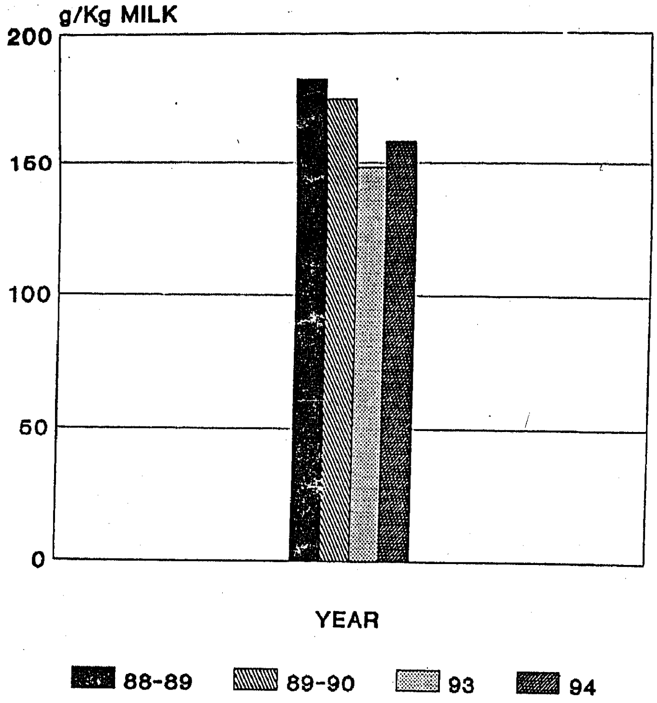

Figure 6. Methane production by Indian ruminant per kg milk production based on milk production and animal population.

0.210 and 0.388 Tg/year. While WRI[1] reported 10.00 million tonnes/year, Ahuja[26] reported 10.4 Tg/year. Both the estimates are higher even at 1990 level of animal population. Share of cattle, buffalo, sheep and goat of methane emission is 62, 31, 2 and 4% respectively (Figure 5). Methane production per kg milk varies between 148 and 183 g/kg milk (Figure 6) based on milk production and methane production estimate of the last 6–7 years. However, it cannot be 240 g/kg in any situation as reported by Aneja[27].

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Dorothy Crowfoot Hodgkin – An introduction to her work and personality

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I had intended to begin this preface by declaring that Dorothy Hodgkin is one of the most remarkable woman scientists of the century. On thinking it over, however, I started to have doubts about the exact phrasing. I realized that the intended tribute might be interpreted as containing a slightly condescending element – quite the opposite of the impression I wanted to convey. Having been brought up in a past era, I am never sure whether compliments of this kind, with the gender classification included, are 'politically correct' or not, whether the restrictive adjectival 'woman' will be interpreted as augmenting the praise, detracting from it, or as neutral, as a simple matter of fact. I trust that readers will accept it as I intend it; as essentially neutral with a very slight element of augmentation, because, after all, in our times and even more when Dorothy was young, it was more difficult for a talented woman to become a scientist than for an equally talented man. In any case, Dorothy belongs in a small group of women who have changed the face of science, as will be abundantly clear from the papers in this collection. The early work in small-molecule crystallography developed naturally into macromolecular crystallography; for me, the landmarks are cholesterol, penicillin, vitamin B_{12}, and insulin, with excursion into the surrounding areas.

In an earlier essay in the volume commemorating Dorothy's 70th birthday, I tried to explain why I thought she had a special place in 20th century science, chemistry in particular. In the first place, she was perhaps the foremost proponent of the idea that the structure determination of complex molecules was more a matter for X-ray crystallography than for traditional chemistry. This may seem commonplace today, but it was not always so. Dorothy not only propounded this idea, she also showed how it was to be put into practice. This was before the advent of nuclear magnetic resonance methods, which soon found a place in nearly every chemistry laboratory to check structural assignments of molecules and to fit in missing details where these were not known. The combination of spectroscopic and diffraction methods helped to liberate chemistry from the burden of structure determination and allowed its practitioners to apply their imagination and ingenuity to advance into new areas. I take here the liberty of defining molecular biology as one of these areas. Of course, Dorothy's work was not done in isolation and owed much to the example and inspiration set by her contemporaries: Bernal, Bijvoet, Pauling, Perutz, Robertson, and others.

Dorothy grew up at a time when chemists argued and even quarreled about the structures of natural products. At meetings of older natural product chemists, one can still hear nostalgic accounts of how A showed that the structure of some compound deduced in B's laboratory was incorrect; a year later B turned the tables on A by showing that the revised structure was still in need of further correction. Reputations could be bruised, feelings could be hurt, and enmities could be created. In contrast, structures determined by X-ray crystallography rarely led to quarrels. They had a satisfying impression of definiteness about them, in contrast to those inferred from chemical reactivity patterns. Molecules were revealed to correspond to objects of definite size and shape, not just intellectual constructions designed to explain chemical reactivity. The price to be paid was that in the course of determining the structure of a compound by crystallography, no new chemistry was done, nothing new was learned about the chemical reactivity of the compound in question. On the whole, however, the liberation of chemistry from the burden of structure determination has opened the way to undreamt advances in synthetic chemistry. Freedom from the task of structure proof has meant freedom from the restriction that a synthesis of a given target molecule had to proceed by steps of known reaction type. Thus, synthetic strategies no longer need to follow along well-established lines but can be of an outspokenly exploratory nature.

In this development, Dorothy's contributions are distinguished by several characteristics that led to her being acknowledged as the leading crystallographer in the field of natural product research. One was her unerring instinct for sensing the most significant structural problems in this field. These problems were usually the difficult ones, the ones that most scientists with any sense would tend to avoid as being insoluble. This brings us to the second characteristic, her audacity in tackling problems that seemed virtually hopeless; I remember the early days of the work on vitamin B_{12} and on insulin, it seemed to me at the time that it would never lead anywhere – but then I am a pessimist and give up all too easily. Dorothy did not give up, and the third factor in her personality was her perseverance to keep going when others would have resigned. She was an optimist in the sense that she was convinced that something would turn up, some new advance that would lead to a solution to the apparently insoluble problem. And she was often right. The vitamin B_{12} work, and even more the insulin work, could never have been completed with the technical facilities that were available when these researches were begun. But the needed improvements in computational possibilities and in the techniques of structure analysis happened, and, as they became available, Dorothy was ready for them, she could put them to immediate use. And then, perhaps most characteristic of all, there was the remarkable skill, experience, and imagination she applied in order to put together the pieces of the puzzle once they began to take shape. As David Phillips has written, Dorothy was the arch-exponent of interpreting Fourier maps: 'Anyone who has seen her at work, surrounded by carefully drawn maps and absorbed in their interpretation, has been privileged to witness an artist in action as, with incomparable insight, she *turns them into shapes, and gives to airy nothing, a local habitation and a name*'.

In this context, it may not be out of place to describe some of my own personal recollections of Dorothy and of her laboratory during the mid-1940s. Towards the end of my doctoral work with J. Monteath Robertson at Glasgow, there were rumours that the structure of penicillin had been established by crystal structure analysis in Dorothy's laboratory in Oxford. This had been achieved, so it was said, in the absence of a known chemical formula for the molecule, and even, it was hinted, in opposition to the conclusions drawn from the chemical evidence. This seemed like a miracle. At any rate, it was obvious to me that such an accomplishment could not have been carried out by the methods we used in Glasgow. For our trial-and-error methods we needed a well-defined molecular model. Although Robertson himself had shown the power of isomorphous replacement in crystal structure analysis 'heavy atom' methods were not current among his students. We had heard of the Patterson function, but the only applications we knew were for very simple structures. We could prove that it would not work in more complicated cases. We were conditioned to think in terms of two-dimensional projections rather than in terms of the three-dimensional structure itself. I read some of Dorothy's published papers, especially the one (with Harry Carlisle) on the structure analysis of cholesteryl iodide, involving the use of three-dimensional diffraction data. I saw that three-dimensional methods were essential for the solution of complex organic structures and decided to go to the Oxford laboratory to learn how to apply them in practice.

In the late spring of 1946 I wrote to Dorothy Crowfoot Hodgkin at the Chemical Crystallography Laboratory, University Museum, Oxford, to ask about the possibility of doing post-doctoral research with her, and I also wrote to the Carnegie Trust to ask if the research stipend I received to support my work with Robertson might be extended for a period to allow me to study with Hodgkin. Dorothy wrote me a friendly letter back, and the Carnegie Trust replied that it was prepared to support me in the way I asked. That summer I went to Oxford for a day or two to discuss details of my forthcoming stay.

Some memories of my first meeting with Dorothy are still quite vivid. At the Porter's Lodge of the splendid, neo-gothic University Museum I was directed to her room, just across from the collection of life-size skeletons of prehistoric animals, including Tyrannosaurus Rex with its fearsome jaws. When I knocked on the door, punctually, at the agreed hour of the afternoon, Dorothy greeted me with her serene, pre-Raphaelite smile and explained that she was just concluding a tutorial. As the two Somerville students were preparing to leave, one of them enquired politely, 'And when should we come back for our next tutorial, Dr Hodgkin?'. Dorothy seemed to consider the question with great concentration before replying, 'Tuesdayish.' 'And at what time should we come?'. 'Threeish'. This was my introduction to a way of expression that was quite characteristic of her. Studied vagueness? It may have been studied, but it was not vague.

In October 1946 I moved to Oxford with my modest stipend from the Carnegie Trust. The Chemical Crystallography Laboratory was housed in a set of rooms in the Museum. The experimental facilities, X-ray tubes, X-ray cameras, microscopes, etc. were in a kind of dungeon, enclosed by massive stone walls, close to the northern entrance to the Museum. Hot water pipes ran through the middle of the darkroom, resulting in quite extreme ambient temperature changes, depending on the hot water supply. The room where I had met Dorothy was indeed her office but it also housed her whole research group, including at the time Barbara Rogers-Low, the mainstay of the recently completed penicillin work, later Professor of Biochemistry at the Columbia University Medical School, and Gerhard Schmidt, later Professor of Structural Chemistry at the Weizmann Institute and founder of modern solid state organic chemistry (sadly, he died far too early, in 1971, but the school which he built in that subject at the Weizmann Institute is still thriving). Perhaps we were half-a-dozen in all. To accommodate this group was a large central table, plus several smaller desks round the walls. And above the grand old-fashioned mantelpiece stood an engraved inscription from Hippocrates to remind us of the transience of our life and the hazards of our craft:

ΒΙΟΣ ΒΡΑΧΥΣ. ΤΕΧΝΗ ΜΑΚΡΗ. ΚΑΙΡΟΣ ΟΞΥΣ.
ΠΕΙΡΑ ΣΦΑΛΕΡΗ. ΚΡΙΣΙΣ ΧΑΛΕΠΗ

At the southern end of the building was the realm of Dorothy's senior colleague, Tiny, otherwise Herbert Marcus Powell, University Reader in Chemical Crystallography (Dorothy's title was then, I think, Demonstrator). This domain comprised a small chemistry laboratory and workshop (where Frank Welch, Tiny's factotum, repaired broken equipment and generally cared for the well running of the laboratory), besides a small lecture theatre that provided office space for Tiny and his co-workers. This was the time when the Powell group was investigating the crystalline inclusion compounds formed by hydroquinone, work that led to Powell's important concept of clathrate compounds.

Another temporary member of the laboratory was Margaret Roberts, a final-year Somerville chemistry student. For her Part II research project, Dorothy assigned her the task of helping Gerhard in his attempts to prepare and crystallize derivatives of gramicidin S, an antibiotic of then unknown structure, now known to be a cyclic decapeptide. Margaret Roberts did not achieve much fame as a structural chemist, but she went on to secure a place in British, European, and world history as Margaret Thatcher.

From time to time, interesting visitors turned up at Dorothy's laboratory. Lawrence Bragg and J. D. Bernal came every few months to hear about our progress. When Bragg came he usually seemed to have forgotten most of what one had told him on his last visit, so one had to explain everything again from scratch. He was not interested in details and was probably thinking of more important matters. Sometimes he came out with a penetrating question about the fundamentals of our craft. Bernal remembered everything he had been told, he asked precise questions and was quick to see connections between one piece of work and another. One day, Max Perutz telephoned to ask whether I would be interested in coming for a day to Cambridge to give a seminar on my Glasgow work. As I recall, the seminars were known as Space Groups in those days. What a fantastic offer! I would actually get my travel and accommodation expenses paid for a visit to a place I had always wanted to visit anyway! No doubt Dorothy had hinted to Max, or to Bragg, that I would greatly appreciate such an invitation.

I should also be grateful to Dorothy for suggesting to Bragg in 1955 that I would welcome an offer to join the research group he was building up at the Royal Institution in London: and again, a year or so later for suggesting to Leopold Ruzicka that I might be a suitable person to build up a new research group in chemical crystallography at the Swiss Federal Institute of Technology in Zurich. Ruzicka had been one of the first to see that X-ray crystallography and other physico-chemical techniques could solve molecular structural problems that would defy traditional chemical methods. He had been impressed by the penicillin work and again, a few years later, by the vitamin B_{12} structure, which, he saw, could never have been elucidated by him or by any of his rivals. He was a great admirer of Dorothy and was prepared to take her advice. So it came about that I went to Zurich in 1957 and have stayed there ever since. Of course, no one dreamt then that vitamin B_{12} would lead to another link between Dorothy and the ETH organic chemistry laboratory.

In my opinion, the B_{12} structure is Dorothy's greatest achievement. Although Dorothy herself would probably give first place to the insulin structure, which had preoccupied her almost from the beginning of her career, my preference is different. By the time the insulin structure was actually solved in 1969, the structures of several other proteins had been elucidated. Although we were all aware of the significance of the insulin work, we had become accustomed to seeing the structures of other, still more complex protein structures, and we were anyway more or less expecting the insulin structure to emerge. It did not bowl us over. The B_{12} structure was different. It was of a level of complexity that had never been seen before among natural product structures, and it was the opening into a new world of possibilities, as aptly expressed in 1977 by my colleague Albert Eschenmoser:

'When Hodgkin announced the complete structural formula of vitamin B_{12} in 1956, it was clear that this natural product presented an ideal objective for organic synthetic research. It is a compound of great biochemical significance. Its molecular architecture is complex and had not previously been encountered in natural products chemistry. Its structural nucleus had resisted elucidation by means of chemical methods of degradation and had been solved by X-ray crystallographic analysis. The synthetic investigation of vitamin B_{12} would involve a host of new problems in the realm of planning and method and would link 'X-ray island B_{12}' with the mainland of chemical experience. Vitamin B_{12} provided an opportunity to extend the frontiers hitherto established by organic synthesis in the area of low-molecular-weight natural products.'

I am probably not alone in looking back at the period before direct methods, before modern computers and diffractometers, at a heroic time with Dorothy as one of the heroines. The goals then, penicillin, vitamin B_{12}, insulin, hemoglobin, myoglobin, seemed almost impossible to attain, and when they were reached they revealed the secrets of nature at higher and higher levels of complexity. Of course, in structure research, as elsewhere, every age is a heroic time, W. H. Bragg attempting the crystal structures of naphthalene and anthracene in 1921 'in order to discover, if possible, some way of handling the complex molecules'; Perutz attempting to derive information about the structure of hemoglobin from three Patterson projections; Watson and Crick attempting to guess the structure of DNA by speculative model-building using stereochemical arguments – and even succeeding. In more recent times we have seen the structure of the photosynthetic reaction centre of a bacterium, providing a structural basis for electron transfer in biological systems – who would have thought it possible! And, looking, into the future, in a few years we may have the structure of the ribosome, the protein manufacturing factory. Yes, we live in marvelously exciting times. And the volumes containing Dorothy Hodgkin's collected papers may serve as a monument to those times. One hopes that they will not only be of interest to historians of science, but also even more that future generations of budding scientists will find in them inspiration and courage to come to terms with whatever problems they may have to face.

Forty years' friendship with Dorothy

Max Perutz

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> *Example is not the main thing in influencing others, it is the only thing.*
>
> —A. Schweitzer

THE four people who took an interest in my early X-ray work on haemoglobin were J. D. Bernal, W. L. Bragg, D. Keilin, and Dorothy. Bernal would listen intently, make some profound comments, and then suddenly sweep off with the air of having to do something far more important. Bragg would discuss the interpretation of my X-ray patterns, but he knew no protein chemistry and not taken any X-ray pictures himself for many years. Keilin was a biologist, and X-ray crystallography was a closed book to him. So whenever I obtained exciting new results, or was disheartened by the persistent lack of them, I would take the now extinct branch line from Cambridge via Bedford to Bletchley and hang around at that dismal junction until the ancient rattly train set off on its many stops to Oxford.

Once arrived, I made for Ruskin's Cathedral of Science – the University Museum – walked past the skeletons of extinct species populating its nave to the darkest corner, and descended the stone stairs to Dorothy's crypt-like office, where she laboured on the structure of life in a place that was, but for her vitality, quite dead (Figure 1). Her tables were piled high with structure-factor and Fourier calculations; there were viewing boxes for looking at X-ray pictures. Her X-ray and dark rooms were adjoining. The gothic window was high above as in a monk's cell, and beneath it there was a gallery, reachable only by a ladder, on which stood a table with Dorothy's polarizing microscope. To mount one of her precious crystals of penicillin, Dorothy would climb up there, stick the crystal to a thin glass fibre, stick the fibre to a goniometer head, and descend again, clutching her treasure with one hand while holding on to the ladder with the other. I don't think she ever lost a crystal.

For all its gloomy setting, Dorothy's lab was a jolly place. As Chemistry Tutor at Somerville she always had girls doing crystal structures for their fourth year and two or three research students of either sex working for their Ph Ds. They were a cheerful lot, not just because they were young, but because Dorothy's gentle and affectionate guidance led most of them on to interesting results. One exception was a certain Margaret

Figure 1. Dorothy when I first knew her.

Roberts whom Dorothy asked to have a shot at gramicidin S, a cyclic decapeptide which Dorothy may have, hoped to solve more easily than proteins. Perhaps Margaret Roberts' failure contributed to her decision to turn to law. When she came to visit our laboratory as Prime Minister some 35 years later, I showed her the structure and told her that it had not been solved until the late seventies when Michael Woolfson's direct methods finally cracked it. However, this consoling piece of news did not make her revert to a career in X-ray crystallography. Despite their political dissension, Dorothy and Margaret Thatcher have maintained a warm regard for each other through all these years. We looked at their latest organic structures and argued about the meaning of our Pattersons of insulin and haemoglobin until it was time to go home to her house in Bradmore Road.

Some women intellectuals regard their children as distracting impediments to their careers, but Dorothy radiated motherly warmth even while engaged in writing crystallographic papers. Concentration comes to her so easily that she can give all her attention to a child's chatter at one moment and switch to lattice transformations the next without any sign of strain. Once she helped her three-year-old son search for a lost toy when he said: 'It must be somewhere; it can't be nowhere.' Not surprisingly he became a topologist. Later in the evening Dorothy's husband, Thomas, would appear if he was not away lecturing to Workers' Educational Association and he would keep us all in fits of laughter over dinner. Afterwards I would discuss proteins with Dorothy until it finally dawned on me that I was exhausting her and that it was time for bed. On Sundays we would go walking or swimming in the Isis. Many years later the two families, six Hodgkins and five Perutzes, went on holiday together to the Austrian Lakes. Once when we had to take shelter in a storm Thomas kept on inventing such good games and stories that he made us feel sorry when the downpour stopped.

Sometimes Dorothy invited me to Oxford to see her latest results. There was cholesterol iodide. She and Harry Carlisle found the iodine positions from a Patterson and used them to calculate the signs of the (*h0l*)s of the monoclinic crystals. The resulting projection showed the molecule well resolved. To determine its stereochemistry, Harry then recalculated the Fourier along a set of lines drawn through each peak perpendicular to the plane of projection. This Fourier was again done with phase angles based on the iodine positions alone, which imposed a false centre of symmetry on the map. With careful stereochemical reasoning and insight, Dorothy picked out the correct one among the two alternative atomic positions indicated for many of the atoms and brought to light the first complete stereochemically correct formula of a sterol. Then came penicillin. Dorothy writes that its revolutionary β-lactam structure had actually been the first proposed, but Robert Robinson was strongly against it because β-lactam rings are generally stable, unlike penicillin, and J. W. Cornforth, then a post-doctoral fellow, supported Robinson's view at an early stage, saying: 'If penicillin turns out to have the β-lactam structure I shall give up chemistry and grow mushrooms'. Luckily he did not carry out his threat and won the Nobel Prize for Chemistry eleven years after Dorothy. The difficult step in the initial structure analysis was to pick out the right lumps of density representing one molecule. Dorothy had a shot at it and then asked others, including myself, to find it. Her first guess proved correct, and further refinement brought to light the β-lactam ring. She writes: 'It was a nice day when we could set up the model first precisely in three dimensions and ring up our friends to come and see what penicillin actually was.'

It was the same with vitamin B_{12}. Anyone else looking at her first Fouriers, derived from the cobalt phases alone, and later from the cobalt plus selenium phases of a selenocyanide derivative, would have attributed the close approach of two lumps of electron density to experimental error, but Dorothy combined a firm belief in the significance of even very blurred features of electron-density maps with a profound flair for the chemically reasonable, and she concluded rightly that the lumps represented rings linked directly rather than through a methene bridge as in porphyrins. She recalculated her phases from the positions of the cobalt plus the atoms of what she later christened the corrin ring, with the result that the next Fourier revealed much of the vitamin B_{12} molecule in outline.

But was it right? On 28 December 1954 Dorothy wrote to me (Figure 2):

> We've had one very nice development on the B_{12} front. Lester Smith fed dichlor-benzimidazole to his bugs and got them to synthesize a chlorine substituted vitamin B_{12}. We had encouraged him to make the dibromo compound but he had difficulties with making the dibromobenzimidazole – and anyway we find that we can see what we want without it. On the very first photograph we took of the dichloro compound there were recognisable small intensity changes on which I straight away did a nice little calculation. It runs like this.
>
> | 032 | 042 | |
> |---|---|---|
> | +11 | −16 | F/4 obs. for dry B_{12}; signs on proposed str. |
> | c+10 | c+1 | Changes in F expected on substitution of Cl for Me |
> | c+21 | c−15 | F/4 values ∴ expected for dichloro cpd. |
>
> Observed on first photograph 032 stronger than 042 (both quite weak). The Cl contribution to 032 is almost the maximum.
>
> Most of the reflections have not visibly changed to the eye. But we shall now have to work up the photographs properly – the expected differences are, of course, usually very small. Cl-Me! But it does seem that this very first observation and calculation confirms that we have quite correctly placed the nucleotide – and this carries with it at least one piece of chemical information, the 3-phosphate binding. Also of course it makes me more than ever confident about everything else.
>
> I have been turning my mind to insulin, thinking B_{12} is drawing to an end and I would like to take up insulin seriously and properly. I

> am not very happy about one's power of model building – even with all the information we have. I gather Lindley and Rollett at Cal. Tech. have built an α helix model, I imagine with parts of chains in helices – I have asked for details. But I would like to work it out properly and I think it can be done with distributed heavy atom derivatives – and a number of years! We have one or two ideas on the heavy atom front which we hope to try out in the next few months. I find myself wondering if the biosynthetic approach is a possible one – easier perhaps to think of for haemoglobin than for insulin.

Ever since she took her first X-ray pictures of insulin in 1935, Dorothy continued to think about its structure. On 26 April 1949 she wrote to me:

> I am very much interested that you have taken up the threefold spiral structure. It certainly is a nice one. Kate [Dornberger-Schiff] and I spent a long time once doing sums on insulin intensities, trying to fit a particular form of it into our picture. Actually when I looked back on those four calculations they seemed not unhopeful – and yet I don't somehow feel in my bones that they are right. And I did some time ago a theoretical Patterson of it – which has quite a lot of good features but doesn't, I think, fit for the gramicidin S.

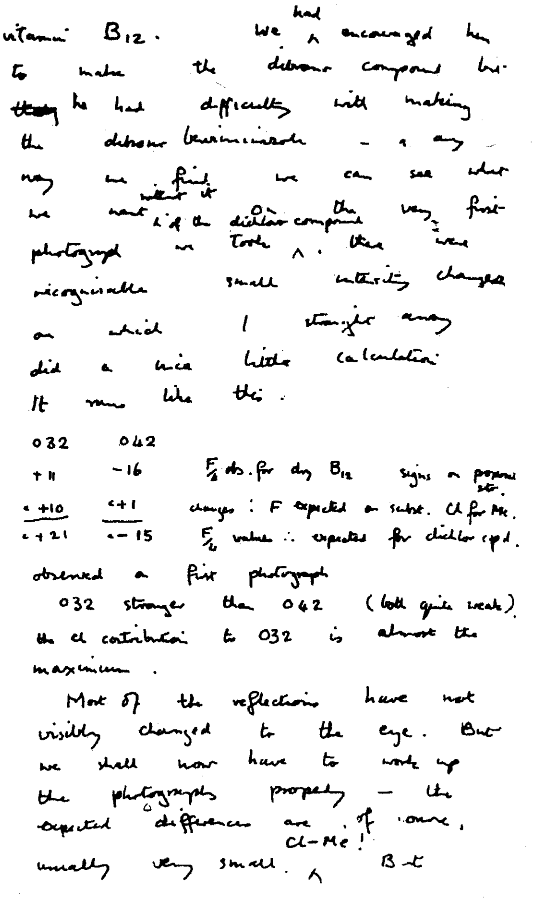
When Sanger had determined the positions of the disulphide bridges in insulin, I sent her a preprint of his paper. She replied on 20 July 1955:

> Thank you ever so much for sending me the insulin paper. It is terribly important – Sanger finds the internal link to be 6 : 11 and this, it seems to me is fatal to simple helical models. Even 7 : 11 I never much liked. Indeed the more one thinks about the geometry of cystine the more one is driven to β type chains (Astbury has said this). I've built a total model for insulin nicely and smoothly and quickly on one of the very oldest schemes anyone has ever had, short folded lengths of β chain (anti-parallel pleated sheet type), in the same configuration that I favour for gramicidin S.

It was another fourteen years before I had a telephone call (Figure 3) from Guy Dodson inviting me to Oxford to celebrate the solution of the insulin structure. I rushed there with a magnum bottle of champagne which someone had given me as a belated present for my Nobel Prize, but when we extracted the cork, all the CO_2 had evaporated and it tasted, as David Phillips remarked in his usual tactful way, just a little peculiar. Then Dorothy showed me that the Patterson of insulin, on which she had spent so much thought, contained near its origin a simple symmetry-duplicated image of the histidines surrounding the central zinc atom which she could easily have interpreted if only she had applied to it her usual faith and courage.

Sometimes I asked Dorothy to visit me in Cambridge. In the summer of 1953 I had just determined the signs of the (*h*0*l*) reflections of horse methaemoglobin by isomorphous substitution with paramercuribenzoate and had calculated the first Fourier projection of the molecule. Dorothy came over at once to admire my map, even though she realized that the many overlapping features of the 55 Å thick molecule would render it uninterpretable. Then she said to me: 'If you could get two heavy atom derivatives, you could solve the structure in three dimensions' and drew my attention to Bijvoet's paper on strychnine in which he pointed to the possibility of solving the phase equation by double isomorphous substitution. This remark sent me off on the next stage of the haemoglobin work. Luckily I did not foresee that it would be another six years before Dorothy would ring me up saying: 'I hear you have a fantastic model of haemoglobin. Can I come over and see it?'.

I felt embarrassed when I was awarded the Nobel Prize before Dorothy, whose great discoveries had been made with such fantastic skill and chemical insight and had preceded my own. The following summer I said



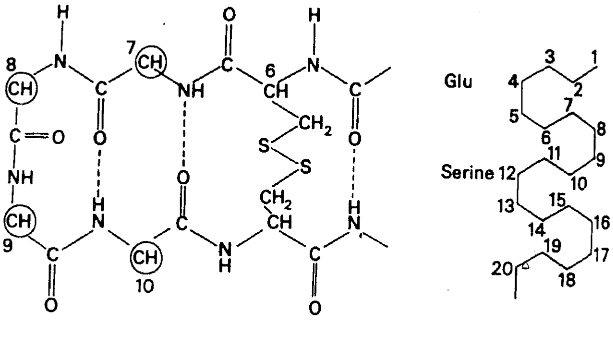
vitamin B12. We had encouraged him to make the dibromo compound but ~~they~~ he had difficulty with making the dibromo benzimidazole – anyway we find we can see what we want what it i.e. of the dichloro compound. On the very first photograph we took. There were recognisable small intensity changes on which I straightaway did a nice little calculation. It runs like this.

032 042
+11 −16 F/4 obs. for dry B12 signs on present str.
c +10 c +1 changes : F expected on subst. Cl for Me.
c +21 c −15 F/4 values ∴ expected for dichlor cpd.

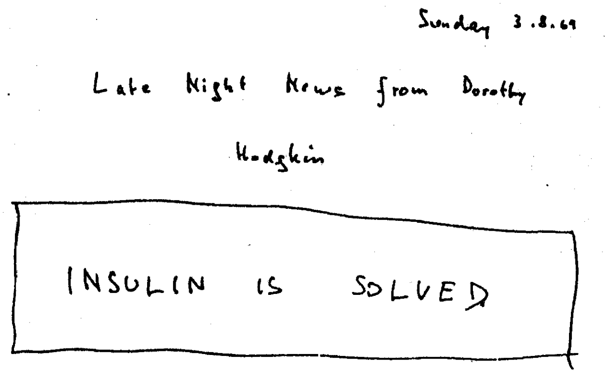
observed on first photograph
032 stronger than 042 (both quite weak).
the Cl contribution to 032 is almost the maximum.

Most of the reflections have not visibly changed to the eye. But we shall now have to work up the photographs properly – the expected differences are, of course, usually very small. Cl–Me! But

Figure 2. A letter written by Dorothy.



Scheme.



Sunday 3.8.69

Late Night News from Dorothy Hodgkin

INSULIN IS SOLVED

Figure 3. Poster at the Laboratory of Molecular Biology, Cambridge, announcing the great news.

as much to the Swedish crystallographer Gunner Hägg when I ran into him in a tram in Rome. He encouraged me to propose her, even though she had been proposed before. In fact, once there had been a newsleak that she was about to receive the Nobel Prize, but it proved false; Dorothy never mentioned that disappointment to me until long after. Anyway, it was easy to make out a good case for her; Bragg and Kendrew signed it with me, and to my immense pleasure it produced the desired result soon after.

'There are certain letters which I dread to open', Dorothy once told me, 'and when I saw one from Buckingham Palace I left it sealed, fearing that they wanted to make me *Dame* Dorothy'. I suppose it would have made her feel like a *femme formidable*, which she so happily is not. When she eventually opened the letter she was relieved that instead the Queen offered her the Order of Merit, which is a much greater honour and carries no title. She received it in private audience on the same day as Benjamin Britten. Once when they were both getting honorary degrees, Henry Moore said to her: 'It's really very good of them to give the OM to a simple chap like me.' I suspect that this remark echoed some of Dorothy's own feelings.

Dorothy Hodgkin and molecular biophysics in Oxford: A fragment of personal history

David Phillips

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THE Laboratory of Molecular Biophysics had its origins at the Royal Institution, London, in the group that Lawrence Bragg assembled there when he became Resident Professor and Director of the Davy–Faraday Research Laboratory in 1954. During the preceding years in Cambridge, Lawrence's main interest had been in the studies of protein crystals by Max Perutz, John Kendrew, and their colleagues, and he had involved himself deeply in the work of the group during the period when very few crystallographers believed that it was likely to be successful[1]. When he left Cambridge in January 1954, however, the tide had turned dramatically. A few months earlier Max, with David Green and Vernon Ingram[2], had shown how the method of isomorphous replacement could be used in protein crystallography and the way seemed open to the detailed determination of protein structures—starting with Max's haemoglobin and John Kendrew's myoglobin. Even the computational problems no longer seemed insurmountable with the growing power and availability of digital computers[3].

Bragg would have liked to take Max and John with him to London to continue the work there, but, at this critical time with so much to be done to exploit the breakthrough, neither was willing to leave Cambridge. But they promised to help Bragg assemble a new group at the Royal Institution and to co-operate closely in its work. At this stage Uli Arndt was already at the Royal Institution, where he had been studying proteins by low-angle scattering with Dennis Riley[4] and developing experimental methods. Early in 1955 he was joined by Helen Scouloudi, who had worked on ribonuclease crystals with Harry Carlisle and now began her study of seal myoglobin. In the autumn, David Green joined the Group from Cambridge and Tony North came from King's College London, where he had worked with J. T. Randall and Pauline (Cowan) Harrison on collagen.

These recruits all had experience of protein work, of one kind or another, but the original team was completed at the end of 1955 by the addition of two more who had not worked directly on proteins. This was Dorothy's doing. Anxious to build up a really strong group at the Royal Institution, Bragg had also asked Dorothy if she would join him there, but she was not prepared to leave Oxford at a time when her family commitments were heavy and the work on vitamin B_{12} was promising well. Instead, with her inimitable knowledge of crystallographers around the world, she suggested that Bragg should ask Jack Dunitz and me to join him. At this time Jack was, as Dorothy puts it, in an American phase of his transatlantic oscillations – damped, as it has turned out – and I was working with W. H. Barnes at the National Research Laboratories in Ottawa, Canada. So it came about that I joined the Group at the Royal Institution in January 1956 and began to work on diffractometers with Uli Arndt and on sperm-whale myoglobin with John Kendrew—who came from Cambridge to see us nearly every week.

Jack Dunitz also joined the laboratory, but he did not work directly on proteins and stayed only until 1957 when he left for Zurich—again at Dorothy's suggestion. To complete the catalogue of people who are now in the Laboratory of Molecular Biophysics and were at the Royal Institution, I must mention Colin Blake, who came from industry in 1960; Louise Johnson, who began as a graduate student in 1962; and Schaffi (R. Aschaffengurg), our incomparable grower of crystals, who collaborated from his base in Reading from 1955 onwards.

In the mid 1950s there was still quite a sharp division in crystallography between those who worked on proteins and those who did not. Many crystallographers persisted in the view that protein structures could not be solved and the protein specialists sometimes seemed to neglect what was going on in general crystallography, regarding the determination of protein structures as a special problem requiring the development of new methods of analysis. This applied neither to Lawrence nor to Dorothy, who made no distinction between proteins and the rest, though their individual attitudes differed in an interesting way. Lawrence liked best to apply the methods he had developed during the early 1920s for the analysis of mineral structures and he was fond of quoting his paper with West[5] on 'The determination of structures with many parameters'—where 'many' means a dozen or so. Accordingly one of my first assignments, when the earliest semi-automatic diffractometer was ready for operation, was to measure the absolute scale of the seal-myoglobin crystals, using anthracene as the reference crystal to measure the intensity of the incident X-ray beam. Bragg's predilection for considering careful measurements of a few individual reflections is illustrated well by his last research paper, on methods for establishing the positions of heavy atoms in protein crystals[6]. Although he had been the first to demonstrate the value of two-dimensional Fourier syntheses in structure analysis[7], he mistrusted Fourier methods, especially Fourier refinement—which elicited somewhat disparaging remarks about boot straps. Dorothy, on the other hand, is the archexponent of Fourier refinement and her papers are full of Fourier maps—many of which seemed meaningless to most other crystallographers when she first described them. (I remember well the scepticism that greeted her early maps of vitamin B_{12} when she presented them at the Stockholm Conference in 1951.) From penicillin[8] through vitamin B_{12} (ref. 9) to insulin[10] the interpretation of electron-density maps of various kinds has been her principal weapon and delight. Anyone who has seen her at work, surrounded by carefully drawn maps and absorbed in their interpretation, has been privileged to witness an artist in action as, with incomparable insight, she *turns them to shapes, and gives to airy nothing, a local habitation and a name.*

Dorothy came often to see us at the Royal Institution, while we contributed to the work on myoglobin and haemoglobin and then branched out into independent studies of β-lactoglobulin[11] and hen-egg-white lysozyme[12,13], and she never failed to convey a conviction that proteins would eventually follow vitamin B_{12} in submitting to the most detailed and refined study. But her support went far beyond encouragement and advice. As early as 1958 she was discussing with Hans Krebs the possibility of our helping to build up a protein-structure group in the Biochemistry Department in Oxford. In the event this idea was not realized, but it led on to John Pringle's successful campaign for the establishment of a Molecular Biophysics Laboratory in the Department of Zoology. The happy result was that most of us who had worked with Bragg at the Royal Institution were able to move to Oxford, when he retired in 1966, to set up this new laboratory. Even more happily, Dorothy, and the flourishing group she had built up to intensify her work on insulin, joined us there.

Fourteen years later the traces of Dorothy's influence can be seen everywhere: our biochemical effort under Robin Offord is largely concerned with exploiting the new knowledge of insulin derived from her studies; the study of triose phosphate isomerase came to us from her, since it was naturally to Dorothy that Stephen Waley turned (as have many others) when he first obtained useful crystals of the enzyme; and everywhere, it seems, there are Fourier maps—as larger and larger structures submit to refined analysis just as she knew they would. When I started work on proteins in 1956 not many people expected that we should one day study the apparent thermal motion of the atoms in them[14] or locate their hydrogen atoms[15]. But I do not think Dorothy is surprised. When we met the other week she was chuckling over two additional water molecules she had just seen in the latest map of insulin. Who knows what she may inspire us to see next?

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Meetings with Dorothy

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Dorothy Hodgkin (née Crowfoot) is a remarkable person and an eminent scientist, who has done much for the glory of her homeland, England, and is well-known for her scientific and humanitarian activities throughout the world. Her personal example and extensive contacts have deeply influenced the development of X-ray crystallography of biological molecules in many countries, including Russia.

In the after-war years, John Bernal often visited the Soviet Union in connection with his scientific work and the world peace movement. On these trips he never failed to visit the Institute of Crystallography of the Academy of Sciences. In 1952 he brought Dorothy with him. She was to speak at the seminar conducted by academician A. V. Shubnikov, the founder and the first director of the Institute. What the audience saw was a woman with a beautiful and intelligent face, who was rather awkward at first, but then went on to speak with mounting enthusiasm about her remarkable work on penicillin and vitamin B_{12}. Whisper went among the audience – it was she who was the first to obtain, together with Bernal, an X-ray pattern from a protein crystal (Bernal, Crowfoot – Pepsin, 1934). Since then, scientists from the Institute of Crystallography and other Institutes of the Academy often met Dorothy both in Moscow and Oxford, and enjoyed her constant attention and support. Her small laboratory in Oxford received postgraduate students from many countries, including those from Moscow.

At that time I was engaged mainly in electron diffraction studies, and later in diffraction of X-rays by high-polymer molecules. But by the end of the sixties I became increasingly more interested in biocrystallography: we started investigations of amino acids, peptides and proteins. Dorothy suggested an interesting topic – deciphering the structure of gramicidin S, discovered by Soviet scientists. Without X-rays, by just considering the chemical formula of this antibiotic, she predicted the conformation of this cyclic decapeptide based on the interbinding of hydrogen bonds. X-ray patterns from this structure (very complex for that time) were analysed by Galina Tischenko – a scientist from the Institute of Crystallography, first in Moscow and then for some time in Oxford with Dorothy. But gramicidin defied solution. It was achieved only in 1989 in collaboration with Dorothy's pupils from York University, and the molecule was just as predicted by Dorothy, but instead of being planar it turned out to be slightly twisted.

The triumph of penicillin, and then vitamin B_{12}, made Dorothy famous all over the world. In 1964 she became a Nobel Prize Winner.

I have met Dorothy many times during her visits to Moscow and on trips to England, at the International Congresses and meetings. The reports she presented at biochemical and crystallographic meetings, e.g. at the VII International Congress of Crystallographers in Moscow (1966), were always brilliant. I have often visited her at her old and rather long two-storey cottage in Ilmington, a small village about 30 miles from Oxford. This is how they lived, the furniture served till it almost fell apart, dinner was always announced by a gong, all rooms were overfilled with books. In Dorothy's husband Thomas' study, the table could be reached only by complicated maneuvering through mountains of books piled right on the floor.

I remember how Dorothy, sitting on the old sofa, talked about her family and the way its members rose to the heights of academic career from their rather ordinary middle-class background – her father was an archaeologist and historian, her husband, an archaeologist too, and Alan Hodgkin – a famous physiologist.

Naturally, their selfless devotion to science was nourished by a powerful intellect, unrestricted by dogmas and paradigms. All the force of Dorothy's mind was directed at what she had set to achieve, once and for all – determination of the molecular bases for life processes. X-ray crystallography became a means to this end. One cannot fail to stress her unvarying persistence in following her goals. As early as 1935, Dorothy started the study of insulin structure. Still, in the world of science, being the first to begin does not mean you will be the first to achieve something. The first structure determinations of proteins already appeared, while the structure of insulin remained unresolved. And then at the VIII International Congress in Stony-Brook (USA, 1969) Dorothy came up to me, her face glowing with excitement: 'Did you hear the news? Insulin is solved'. Everybody was glad for her success, which she shared with her pupils, many of whom have now become eminent scientists. At present many modifications of this remarkable molecule have been studied in different laboratories of the world (first of all, in England) by the method of X-ray diffraction, to a very high resolution of 1.0 Å.

Once after the appearance of the remarkable works of Kendrew and Perutz on myoglobin and hemoglobin, I asked Dorothy if one could make generalizations on the structure of protein molecules. 'No', she replied, 'it's not time yet'. And added thoughtfully: 'If we had at least ten structures, then we could probably make some conclusions'. And what can we say now, when thousands of such structures are deciphered? Basically, the answer is the same – we must go on working. Interaction of proteins with other biomolecules, the mechanism of their action, protein engineering, fighting disease at the molecular level, computer designing of drugs – such are the new problems that have sprung on the basis of earlier solutions. The collective wisdom of the world scientific community points to the directions in which the efforts and finances of their governments and companies should go. While in the first years after the Second World War there were only 5–7 biomacromolecule structure laboratories in the world, now their number exceeds one hundred. Physics was traditionally considered the queen of sciences, but will she probably have to share the crown with molecular biology in the 21st century?

I became convinced soon that Dorothy not only has a deep and astute mind, but a character full of firmness and determination. Her devotion to science is selfless, and she completely abandons herself to work. Her contacts with scientists in England and many other countries, like China, USA, Holland, Sweden, Russia, were most extensive. This has always kept her in good form, scientifically, and ensured the support and assistance of scientists in her work.

Science is definitely Dorothy's true calling, but she is also a humanitarian and a prominent public figure. Her attraction to ancient civilizations of the Orient and Africa is well-known. The contacts between the Russian and English crystallographers, initially established mostly through Dorothy's efforts, grew increasingly wider and firmer. We have traditionally close ties between the scientists from the Institute of Crystallography of the Russian Academy of Sciences and crystallographic laboratories in London, Oxford, York, and Cambridge.

The world's estimation of Dorothy's achievement brought her many awards and honors. In 1976 she was elected foreign member of the USSR Academy of Sciences. In 1982 she received the highest award of the USSR Academy of Sciences – the H. V. Lomonosov gold medal. She came to Moscow for the official ceremony and made a brilliant report at a session of the Academy.

In 1969–1975 Dorothy was the President of the International Union of Crystallography, and I, member of its Executive Committee. I discovered then that for all her kindness, Dorothy is an excellent organizer who could pass important resolutions among her not always agreeing colleagues. In 1976–1988, Dorothy was the President of the Pugwash Movement of scientists for peace, and worked in close contact with her Soviet colleagues.

In 1988 a delegation of the USSR Academy of Sciences came to England on the invitation from the Royal Society and was then received at 10 Downing Street by Prime Minister Margaret Thatcher. After talks at the first floor assigned for official receptions, we were led to the second floor that housed her study and a collection of portraits and sculptures of famous figures from England's history and science (the third floor, as it is well known, is the Prime Minister's private quarters). Above her writing table there was an excellent water-colour of Dorothy Hodgkin. In her college years Thatcher had been her student, but had then preferred a political career to that of scientist. However, even as a Prime Minister she did not break her contact with Dorothy.

There is another excellent portrait of Dorothy Hodgkin at the National Portrait Gallery in London. It shows Dorothy in the midst of scientific research: her wonderful blue eyes shining with excitement, and her hands raised to a model of some complicated structure.

DOROTHY CROWFOOT HODGKIN – IN MEMORIAM

Dorothy Hodgkin and the Indian connection

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Dorothy Hodgkin – as crystallographer, scientist and human being far surpasses most and so it is not easy to write about her many-splendoured personality. Instead, in this essay I shall attempt to present the influence she has had on the growth of X-ray crystallography in India, directly through those who worked with her and indirectly by her travelling all over this country. In such an account, one must be pardoned for some personal element creeping in.

In the twenties, India had developed a fairly strong tradition in X-ray physics. The six-week visit of C. V. Raman to Europe in 1921 greatly changed his research interests. On seeing the blue of the Mediterranean he started on his researches on the scattering of light in liquids which finally culminated in the discovery of what is now called the Raman Effect. His encounter with William Bragg and his naphthalene structure started three lines of research in India. (a) Raman fabricated an X-ray tube and was amongst the earliest to use X-ray diffraction as a structural tool to study liquids. He showed that while in large angle scattering the haloes reflected specific molecular sizes and packing shapes, small angle scattering was directly related to the statistical fluctuation of density in a liquid. (b) Raman knew that Bragg's first structure of naphthalene was not consistent with its birefringence, while the second one was. With this as a cue he and his school launched extensive studies on the optical and magnetic anisotropy of organic crystals to get vital information on the arrangements of molecules in the crystalline state. (c) One of his students, Kedareshwar Bannerjee, was amongst the first to probe into the problem of phase determination by direct methods and for this he used Bragg's data on naphthalene. Unfortunately, in spite of this early lead, the first crystal structure was solved in India using Fourier methods by Gopinath Kartha only in 1951. The Indian Institute of Science (IISc) had great hopes of starting a powerful school of X-ray crystallography when G. N. Ramachandran came back from Cambridge. But he went over to Madras and there he established one of the most renowned Schools of Biophysics. With Gopinath Kartha he solved the structure of collagen.

A vacuum was created in Bangalore when Ramachandran went to Madras along with his entire team. R. S. Krishnan (then head of the Physics Department at the Indian Institute of Science, who had a knack of picking young people to lead new scientific groups) asked me in 1951–1952 to be 'in charge of X-ray crystallography' and attempt to grow a group 'worthy of the Physics Department'. The first problem Venkatesan and I took up was to use the novel idea of multiwavelength anomalous scattering (MWAS) to solve the phase problem and we demonstrated its feasibility (1952–1954). Almost immediately I had to spend a year in the United States with I. I. Fankuchen (Fan) working at Brooklyn Polytechnic. My entire outlook towards X-ray crystallography changed when I attended a one-day seminar held there on the phase problem at which David Harker, David Sayre, Herbert Hauptmann and Jerome Karle spoke. At the end of Karle's lucid presentation I remarked to Paul Ewald who was sitting next to me – 'It seems as though this method is going to work' to which Ewald commented, 'Yes it will, and it will take away the excitement from most of the older crystallographers.' It seemed to me as though most techniques connected with the phase problem: the heavy atom and the isomorphous methods, the Patterson synthesis, the vector superposition techniques, approaches through anomalous scattering and finally, direct methods had been formulated. Many details were yet to be worked out but what really remained to be done was to use all the techniques for actually solving crystal structures large and small. At that time (1954–1955) the structures of DNA and collagen had been determined, but one realized that the approach and intuition necessary to solve such structures were very different from those necessary to solve complex single crystal structures.

I discussed this matter in some detail with Fan (who had wisdom and insight. Incidentally, it was he who shortened my name to Siv). We agreed that if X-ray crystallography were to take root in India or indeed anywhere else it must become a 'useful' tool to chemists, biologists and medical people. The centre of gravity of X-ray crystallography must shift from 'Physics Departments' to others. It was in this connection that he mentioned the name of Dorothy Hodgkin.

I had, of course, heard of her and read her papers. I did have a slight confusion in my mind – her randomly oscillating between the names Crowfoot and Hodgkin. Her range of interests was perplexing. Proteins – pepsin (1934) and insulin (1935); steroids (the famous paper with Bernal and Fankuchen, 1940). The cholesteryl iodide paper (1945) was a classic, from which I learnt how to use the Patterson and the heavy atom methods; penicillin (1949); pernicious anaemia factor (1950), etc. Every paper was a gem in which she used some new technique and made it work. She wrote with such facility that the reader felt that it was all so very easy.

Fan then said that inspired by her mentor J. D. Bernal and through her sheer genius she was demonstrating the power of X-ray crystallography to the world and 'was thus freeing chemists and biologists to do greater things' (although they themselves were resenting it, as children often do). It was later that I realized what she meant when she said – 'There our scientific world ceased to know any boundaries.'

I also met Dorothy Wrinch at Smiths College and Patterson in Philadelphia. Again Dorothy was mentioned with great respect and affection. Two of their comments struck me. Dorothy introduces her collaborators into problems in crystallography which they could never have even dreamt of and persuades them to use techniques that were absolutely new. They would scarcely know that they were going through a regimen of training not only in crystallography but also in scientific attitudes. The other was that Dorothy was such an internationalist that she welcomed having colleagues from every part of the world and always insisted on their going back to their own countries and working there. All this heartened me for the exodus of our better brains had already started and it attained serious proportions later. I felt it would be ideal if Indian crystallographers could hitch their wagons to this star. I started making preliminary attempts but strangely they only crystallized later on.

Venkatesan and cobyric acid

After his work with me on MWAS, Venkatesan took up the structures of a few organic molecules (for I felt that he should become the nucleus in India for taking crystallography to organic chemists). It was therefore fortunate that Jack Dunitz (who was Venkatesan's thesis examiner) offered him a Fellowship to work in ETH, Zurich. In 1961 at Jack's suggestion, Venkatesan joined Dorothy's group and was promptly christened *Van* by her. Dorothy suggested that he work on the structure of the nucleus of vitamin B_{12} – cobyric acid, the orange red crystals isolated from sewage sludge. She also said that while it should not be difficult to solve its structure by the heavy atom method, with his earlier experience in anomalous scattering, 'it may be fun to try the anomalous dispersion method' and with a twinkle in her eye she added 'it would, of course, mean measuring visually the intensities of thousands of reflections'.

Van writes 'It was clear to me that Dorothy was very interested in seeing the power of the Bijvoet method applied to a largish molecule, and so David Dale and I took it up immediately. I enjoyed greatly working on this structure – continually getting valuable ideas from Dorothy, often crucial to the successful application of this method'. All of us were so happy when his work was dealt with in such detail in her Nobel Prize discourse.

'Today our best evidence of the structure of the nucleus of B_{12} comes from the X-ray analysis of cobyric acid. By measuring the intensities of both Fhkl and Fhkl, Dale and Venkatesan were able to assign rather accurate phase angles to 1994 reflections ... in the 3-dimensional Fourier map even many hydrogen atom positions appear as peaks, – leaving almost no doubt of the correct formulation of the chemical structure.'

It was in Zurich and Oxford that Van's complete conversion to organic chemistry took place. 'I consider myself extremely fortunate to have worked in two world-renowned centres of chemical crystallography.... A week before I left Oxford, Dorothy advised me to visit Chemistry Departments in India and make it a point to discuss their chemistry.'

A year of enjoyable activity

On Van's return, I went to Oxford for a year (1964–65) ostensibly to 'look after' Dorothy's doctoral students when she was to be away in Ghana with her husband Thomas. I arrived in Oxford with my wife and three young daughters. Dorothy then suggested I look into a recalcitrant structure – vitamin B_{12} monoacid which Clive Nockolds had been tackling for some time. A subtle error in indexing was detected and from the visually estimated intensities of about 6500 reflections almost 3000 Bijvoet pairs could be accurately phased. We got the immense satisfaction of picking atom by atom from the first 3-dimensional map till the entire structure unfolded itself in all its glory.

Just before she left for Ghana, in a vague manner (which some of us feel she adopts when talking of very serious things) she asked me whether I could look into the anomalous scattering effect Petersen (who had worked with Bijvoet) and Smith had discovered in neutron scattering in CdS. Having lectured only recently to the undergraduates in Madras, I had some familiarity with nuclear physics, reactors, cadmium absorbers (which Fermi used), of Breit–Wigner formalism of neutron scattering and the Kramers–Kronig relations in optical dispersion. I could see that in ^{113}Cd the dispersion corrections b'' and b' would be 500% to 1000% of normal scattering amplitude b_0 (as against 5% to 10% in the case of X-ray scattering); that b' would actually change sign (from say $+5b_0$ to $-5b_0$). Introducing special isotopes ^{113}Cd, ^{149}Sm, ^{157}Gd and ^{151}Eu and choosing an appropriate neutron wavelength is equivalent to introducing a very heavy atom in the structure, a concept completely unknown in neutron diffraction; and finally by using the multiwavelength method one could even solve structures with 5000–10000 atoms! The implications of these ideas were so unexpected that Dorothy took me to talk to J. D. Bernal. He heard me with great patience and said that all my ideas were basically right – but they can become practicable and routine only if the intensities of the neutron beams increased by at least one order of magnitude. The oracle had spoken. In spite of the fact that a few structures were solved using this method, and Dorothy herself put a Cd insulin in the neutron beam the verdict was – 'unfortunately Ramaseshan's suggestion (1964) of using anomalous dispersion of neutrons could not be used to solve the then unknown structure of insulin due to limitations of flux'.

It was wonderful to be with Dorothy's doctoral students, who were an international lot. There was Tony Cooper (USA) who worked on a derivative of fusidic acid and who was one of the earliest to combine photographic methods with automatic microdensitometers and computers for the quick collection and indexing of data. This work, in my view, paved the way to the later sophisticated developments in this field. Mike James (Canada), who displayed all the makings of the outstanding crystallographer he became later, solved a crystal which had a non-crystallographic symmetry in it; Mannan from Pakistan whose thesis I had the pleasure of correcting, Eli Schefter who worked on drug molecules. Tom Blundell who was to join the insulin group later had already started working for his part two – and many others. One was busy but I had enough time to participate in the insulin programme. Working with Guy Dodson – who was the mainstay of Dorothy's insulin team – was always exciting – with the mandatory cricket game one had to play in the lab with a rolled up Fourier map as bat and a crinkled paper as ball. The insulin team consisted of Guy and Eleanor Dodson, Margaret Adams, myself and Dorothy. The major attack of introducing heavy atoms into insulin started then – removing zinc by soaking crystals in EDTA solution and introducing lead and cadmium, testing for isomorphism, collecting data on anomalous dispersion in 2 (or 3) Pb insulin, in 2 (or 3) PCMBS insulin as also in 4 Zn insulin containing HgI_4 (which had to be cooled by blowing cold air as we had done in Bangalore and Brooklyn to prevent the denaturing of the crystal). It became clear to us that having so many strong anomalous scatterers in a cluster was not very useful. It was also at that time that the methods of calculating contribution of the heavy atoms alone using anomalous scattering (and isomorphous) data were evolved. Eleanor started writing the computer programs which played a major role in the final solving of the insulin structure. One also came to the conclusion that the Phillips linear diffractometer (which broke down often giving us cause for much fun and laughter) must be replaced by a four circle one.

It was a magnificent year. Dorothy was awarded the Nobel Prize. As she could not be reached in Ghana by the Nobel Committee we were the first to announce it to her! When she was elected to the 'Order of Merit' we celebrated it in the lab with an old case of Danish beer which lay hidden in the cupboard amongst the Fourier Maps. When lysozyme was solved by David Phillip's group she took us to the Royal Institution. I was flattered when Lawrence Bragg recognized me as the person from Madras who also arranged for his seeing some of the beautiful birds there.

I also knew at the time I left Oxford that cobyric acid and vitamin B_{12} monoacid were really stepping stones for using anomalous scattering to solve the insulin structure. The base camp had been firmly established for the final assault and the clouds were clearing up and Dorothy could see the top of the peak.

Viswamitra and use of the Patterson

M. A. Viswamitra joined me as a research student in 1955. He was one of the most talented of my earlier students, very independent, with remarkable experimental skills and one who could choose his problems with great discernment. It is of him that Dorothy says, 'Viswamitra, – an Indian research worker visiting our laboratory who has a charmed hand as far as crystals are concerned'. It is of interest that the first problem I set him was to grow single crystals at low temperatures by freezing liquids – which he did with great ease. He joined Dorothy in 1965 just before I left Oxford and worked on the structure of thiostrepton – a large antibiotic molecule with a wide spectrum of activity against gram positive bacteria, comparable with that of penicillin. It crystallized in the tetragonal form, which presented many problems. Mitra by changing the solvent got a more convenient monoclinic form with two molecules per unit cell. Even here the determination of the chemical structure was difficult because of the variability of its hydrolysed products and nor was its chemical formula known: No heavy atom derivative could be obtained. The molecule was alleged to have 5 sulphur atoms and their numbers and positions were quite uncertain. Viswamitra and Bryan Anderson boldly attempted to solve the structure using the Patterson superposition vector shift and Buerger minimum function methods. Writes Mitra (rather flatteringly to the present author) 'The vector shift method was rather new at that time. And I acquired a good background in it when I worked with Ramaseshan, my thesis adviser. The confidence and training I had received with him made me hope that the method would prove successful'. And successful it was. Dorothy Hodgkin in her celebrated Patterson Memorial Lecture gives this as the supreme example of the power of the Patterson technique. Says she: 'They set out with the three-dimensional Patterson drawn on transparent sheets and superposition from every peak in the map (was made) looking for 4 to 5 five-membered rings which should be thiozole rings . . . I never knew how in the end the structure was solved. The first map that Viswamitra calculated was one of the ones that make me laugh, . . . if it does not make me cry – to think of. But still in the end they got the structure out – a very remarkable feat indeed.'

Mitra also worked on ferroverdin, the green iron-containing pigment (isolated by Chain and his group) which showed extremely interesting magnetic and chemical properties (*Nature*, 1969, **224**, 589).

Vijayan and insulin

Immediately after Mitra left Oxford, M. Vijayan joined Dorothy in January 1968. Vijayan's thesis adviser was Viswamitra. Dorothy was one of the thesis examiners and I was also on the panel. Dorothy informed me that she would like a crystallographer to join the insulin group as Margarat Adams was finishing her thesis and would almost certainly leave. With this new addition she hoped to make a serious attempt to solve insulin. Therefore, when she was to be in India (in 1967) she would like to meet Vijayan. Dorothy met Vijayan and offered him a position. Writes Vijayan: 'Most of my well wishers asked me *not* to join the insulin group. Dorothy had been working on insulin for decades and the solution was not at all in sight. But I decided to work on insulin, primarily as I wished to start a protein group in India; and I could not imagine any other place than Dorothy's to learn the subject. I joined in 1968. The group finally consisted of Guy and Eleanor Dodson, Tom Blundell and myself – the division of labour was not watertight – everybody did a bit of everything – not perhaps the most efficient way – but an excellent strategy in the long run, as each worker far from being a narrow specialist became a well-rounded protein crystallographer. Tom and Guy prepared many heavy atom derivatives. My task was to collect the data on the newly installed four-circle diffractometer and Eleanor did much of the computing. Dorothy decided that the anomalous scattering approach would be the most effective route.' Eleanor and Vijayan developed many techniques for the effective use of this method. All these are described in their paper (*Acta. Cryst.*, 1971, **B27**, 2402). When the structure was clearly coming out there arose a problem. Vijayan had promised Kalyani that he would come back early in 1969 to India to marry her. To keep the team intact – 'the mountain was brought to Mohamed' – Kalyani was brought over to Oxford on a fellowship and their wedding took place at the height of the fever of insulin being solved. Obviously there was no time for a honeymoon. Instead Vijayan had the thrill of seeing along with Guy in the first phased map of insulin, the stretch of alpha helix (10–19). Wrote Vijayan: 'Insulin was solved and Max Perutz came to Oxford with a huge bottle of champagne. Although the champagne turned out be somewhat flat, the atmosphere was nonetheless intoxicating.' The structure was announced at the IUCr Congress at Stony Brook (in August 1969). It was characteristic of Dorothy to ask the youngest member of the team – Tom Blundell to present the structure. I happened to be there to see the crystallographers marvel at this achievement and the stamina and persistence of Dorothy Hodgkin over 34 years. None of Dorothy's colleagues in the insulin group which was involved in the final solution of the structure in 1969 was born when the first photographs of insulin were taken by her (*Nature*, 1935, **135**, 591). Says Vijayan, 'Although one was pleased and excited when insulin was solved – that in some sense one was participating in a piece of history dawned on me only much later'. At Stony Brook Dorothy reminded me of our meeting Vijayan in Bangalore just two years previously. 'I feel so happy that Vijayan did join the insulin group. In Sanskrit I am told Vijayan means the Victorious: Truly he did finally bring us victory'. A rich tribute indeed.

Dorothy in India

Dorothy has visited India often, twice as Raman Professor of the Indian Academy of Sciences. She gave the Gandhi Memorial Lecture and the Azad Memorial Lecture and spoke at the Pugwash Conference in Madras. There was a time when we thought she could spend a part of each year in India as a visiting professor to escape the winters of England which greatly affected her arthritis. But this was not to be, as Thomas's health would not permit it. Unlike most distinguished scientists who came to India just to attend a conference or two and to visit a few very sophisticated centres of research, Dorothy travelled to every nook and corner of India, inaugurated conferences, gave tens of keynote addresses, scores of lectures, seminars to audiences – large and small, visited laboratories, universities and colleges, discussed with groups and individuals. On one memorable occasion she gave an unforgettable lecture when Thomas was seriously ill and was actually fighting for his life. She travelled to Cochin and Trivandrum in Kerala, visited the southern tip of the subcontinent where the three seas meet, Madurai (the great temple city), its university and its excellent biology department, Madras, Bangalore (IISc, RRI, NAL), Hyderabad, Bombay (BARC and TIFR), Delhi, Lucknow, Cawnpore and Calcutta. We travelled to Gauhati in the eastern corner of India where she inaugurated a Congress of scientists and addressed thousands of students and had at least 5 to 6 engagements each day for 4 days. We then drove to Kaziranga on the evening of the Krishna festival. In the twilight each of the hundreds of villages we passed were lit up with oil lamps and were celebrating the festival – with music and dance – she said that it was so beautiful, so graceful and so enchanting. At Kaziranga, the home of the Indian one-horned rhinoceros, she spent a restful day seeing many of these incredible creatures for the first time and also thousands and thousands of birds. The day we left Assam the famous/notorious 'Assam Agitation' started and myriads of citizens and students took to the streets. She was not perturbed at all, spoke to the students, although I was (on her behalf). We then went to North Bengal, famous for its Naxalite movement. At its epicentre she addressed the students and staff of the Siliguri University which was courageously starting crystallographic research. She gave many talks and had many discussions. I knew she was straining herself too much – 'How can I say no when so many of the young and enthusiastic are eager to listen to me, talk to me and even just see me', she said.

She had never seen the 'Abode of Snow' (the Himalayas) from close quarters. We motored up to Darjeeling and saw from there the snow-covered mountains forming a colossal amphitheatre. I was getting worried about her health. Even so, at 4 AM we drove miles and stood on top of Tiger Hill. Before the sun rose, its rays hit the world's highest peak, Mount Everest, which stood there in solitary splendour against a dark background: In succession the lower peaks, hundreds of miles apart, all above 25000 ft appeared one by one – the heavenly spotlight picking them out – pink dressed ballerinas that adorned the magnificent mountain stage – till finally the great 'Kanchanjunga' exposed herself in the yellow fiery light – truly a pyramid of gold. When the sun was finally up Dorothy spoke: 'It was truly magnificent. I would not have missed it for the world – Thank you, Siv, for not cancelling this visit.'

By that time I knew she was quite unwell. We flew to Calcutta and then on to Delhi. I shall never forget the help I received from Anna Mani, one of our distinguished women scientists. She arranged for two young doctors ((Ms) Sharma and her husband) to examine her and later (Ms) Padmavathi, one of the best heart specialists we have, who after careful examination declared that one need not be too alarmed. 'The pills and some rest will quieten the fibrillation of the heart and then she can board the plane for England.' For almost ten days I stayed with her at the IPCL guest house (courtesy its Chairman, S. Varadarajan). It was then that I heard about her childhood, her father in Sudan, her mother the textile expert – about her sisters and nephews, her first interest in science. Her chance meeting with Lowry and Joseph which took her to Cambridge and to J. D. Bernal – her interest in insulin and penicillin, steroids, B_{12}, etc. Her meeting with Patterson, Kathleen Lonsdale and a hundred others. It was all so exciting and I felt like writing her biography.

I cannot end this section without a few words about Thomas Hodgkin. I knew him well and we spent hours together conversing and arguing. He had a sharp tongue – but there was absolutely no malice. A scholar with a deep knowledge of History, with a sense of humour that kept you laughing all the time, a most loveable man. In 1982 we got a letter from Dorothy saying that Thomas died. It was sad. When we saw her next, there was a change, something was missing and she appeared far more pensive and serene. In 1984 Kausalya and I made a visit to the place where Thomas died, Tolon in Greece – a country he loved, whose language and art he respected and from whose mythology he often quoted, the day he died commemorated the day on which Greece was declared independent – that too at a place close by. He came out of the house on a beautiful sunny day and just before he fell down dead he saw the indescribable beauty of the bay of Argolis. Yes, in spite of our great sorrow one could not but envy him.

Growth of crystallographic laboratories in India

Dorothy was unhappy about Gopinath Kartha – one of our best crystallographers, settling down in the United States. When Van said that this was probably because of the lack of facilities in India she told him with her characteristic modesty that the greatest satisfaction she derived was when she carried out her investigations under the most difficult and adverse circumstances. Again to Vijayan she said 'Modern facilities are often necessary; however it is really the people who make science'.

Each one of her Indian collaborators knew that she would like them to go back to India, work, and establish groups and laboratories. And they to a large extent succeeded; perhaps they were driven by the fact that they could not fail her. In this process one must be grateful to Satish Dhawan the farsighted Director of IISc who not only brought G. N. Ramachandran back to Bangalore but also appointed many of the younger crystallographers into different departments.

Van joined the Organic Chemistry Department and established one of the better X-ray laboratories for organic and drug chemistry. He tells us the story of when he heard Leslie Leiserowitz of Israel giving a lecture in solid state photochemistry in Oxford, how he told Dorothy that he would like to go to Israel to get exposed to this area. Dorothy's immediate reaction was 'Van, you don't have to go there. You can do it all in India itself'. This gentle but 'firm' advice had its effect. He did start studies of solid state photochemistry along with a very bright young photochemist Ramamurthy who had worked earlier on liquids. He always wonders whether I, who had heard the earlier conversation, had anything to do with getting Ramamurthy to Bangalore when I was director of IISc. Venkatesan describes how they had to preorganize crystal packing so that they got the photoproducts they required, a process Schmidt called crystal engineering. This team did much work on coumarins, dynamic photochemistry and gas solid reactivity of thioketones, host systems with cavities and reaction in cavities. Gautam Desiraju who was at IISc left for Hyderabad and is doing good work in this field there.

Viswamitra wrote: 'I was in Dorothy's lab from 1965 to 1968 and this had a much wider significance to me: It led to my fascination for biological molecules and later to my commitment for work on such molecules.' Mitra established at the IISc a reputed school for the study of oligonucleotides. He and his school pioneered studies on the conformational differences between two nucleotide species because of the possible relevance to DNA and RNA structures. It is now agreed that the concept of the DNA double-helix having sequence-dependent variability in its structure came after Mitra's laboratory determined the crystal structure of the tetranucleotide d_p A–T–A–T. The idea of taking up this sequence came from a discussion Mitra had with Francis Crick. Mitra also established a close collaboration with Olga Kennard in Cambridge. In much of this work he again used the Patterson superposition technique. His pursuit of DNA crystallography is continuing and he has with his 'green fingers' crystallized many decanucleotides and has found an alpha DNA structure in some of these.

Vijayan has established a school of small molecule and macromolecular crystallography. Their work on biomolecular interaction, including their implications for chemical evolution and the origin of life is now recognized. His centre has been functioning for some time and it carries out the structure analysis of lectins (peanut, jack fruit, and winged bean). Once when I was able to get funds for the crystallographic groups at IISc I asked Dorothy if some type of organization or direction is called for. Her reply was, 'It is people who matter, once the proper choice has been made it is best to leave them alone and let them organize themselves'. It was sound advice and it paid dividends.

It must not be thought that crystallography in India is confined to those who actually worked with Dorothy. Her inspiration has gone down to the next generation and to the next. I invited Venkatesan's student M. R. N. Murthy (who was working with Michael Rossman in Purdue) to come to the IISc. He came back readily and the first virus structure from his group has appeared recently in *J. Mol. Biol.* Extremely good schools of protein crystallography, neutron crystallography and high pressure crystallography, have been established in Bombay by one of our noted scientists R. Chidambaram (again a product of the IISc and a contemporary of Van and Mitra); his protein group is now being led by T. S. Kannan, Mitra's student. Applied crystallography, has been established in NAL (A. K. Singh and Kalyani Vijayan). Good groups have sprouted out all over the country – not accidentally at places which Dorothy visited. Yes, India owes much to Dorothy and the world at large may never suspect the yeoman service she did for us.

Dorothy's graciousness and humanity

After my return from Oxford I often wondered whether I had justified the confidence she had reposed in me by her inviting me there. I was truly surprised when she wrote some time later. 'In Oxford, we first began to know something of his qualities when he spent a year in the Crystallographic Laboratory. It might have been a difficult year for my research group. I was a great deal abroad. In fact, it was a marvellous experience for them being guided instead by Sivaraj Ramaseshan with his great learning and critical understanding. Complicated structures were solved under his hand, mistakes put right and new approaches to structure analysis developed. It was not surprising to find that the whole group had become his devoted friends and admirers – and had developed great respect for Indian science.' Whether this is true or not is immaterial. It is another example of Dorothy's graciousness and magnanimity; her realizing the need to encourage one from a far-off country where the playing fields of science had not yet been established.

Yes, she is the ultimate in graciousness and kindness. The only manner in which an Indian can describe her is to say that she treated us as members of her own family. The little considerate things she did moved us. Sending a telegram welcoming to Europe my family when we reached Naples by ship from India. Seeing personally that Van had a comfortable place to sit in the lab and one to stay outside; the concern she showed to the families of Viswamitra and Vijayan when they were not well – are just a few of the innumerable examples one could quote. She made us feel that she was giving to each one of us a treatment different from that given to others, that each of us was very special to her. How she found time to look after all her brood, select appropriate presents for each one of them, one would never know. It is good to remember that her brood extended over countries and continents, United States, Canada, New Zealand, Australia, Ghana, Nigeria, Egypt, Sudan, Morocco, China, India, Vietnam and it goes on and on. She became friends with all the children and remembered their names. Once when my wife and I came back to Oxford from London, our children greeted us in the middle of the night shouting, 'Our Dorothy has won the Nobel Prize; we saw it on TV' To 10- and 8-year olds she had become 'Our Dorothy'.

Every time we were in England we visited her and Thomas in Crab Mill in Ilmington – their beautiful long house which seemed at first sight all chaos but which was really a home to all of us. Someone from some corner of the earth was always also there. We could stay there as long as we liked – breakfast, lunch, dinner (Thomas always made vegetarian dishes for us). All sat together and relaxed in the garden; Dorothy showing us some manuscript of hers for an opinion. Thomas having heated arguments on the state of world. Or we would go to dine with Dorothy's remarkable mother-in-law who lived in a beautiful house up the road when she would tell of the naughty things that happened in the early part of the century at the university. Dorothy's greatness does not rest on her having solved cholesterol, penicillin, B_{12} or insulin. True, these are unparalleled achievements. But to many of us her greatness also lay elsewhere which made her spread her science to all parts of the world and also in her concern for humanity. All pay lip services to the cliché, 'We must love humanity'. But Dorothy's method of doing this was like that of Gandhi or Mother Teresa, to have compassion and a personal affection for every fragment of humanity – young and old, wherever and whichever corner of this world it was found in.

Dorothy and insulin crystallographic research in China

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ALTHOUGH only two of us have the privilege to be the authors of this article, we believe that many other Chinese scientists, who know Hodgkin either personally or by her fame as a great scientist, have the same wish to express their respect and esteem. Dorothy Hodgkin is to us a dear friend who has given us help and support in our work. What we express here is only a small part of what Dorothy's many friends in this country wish to say.

One of us, Dong-cai Liang, crystallographer, worked on seeking heavy atom derivatives of insulin crystals in the Department of Zoology, University of Oxford under the guidance of Hodgkin in 1966. 'It was an unforgettable experience in my life, at Oxford with Dorothy' Liang told his colleagues and students. 'There I was, the first scientist from Red China in Dorothy's international laboratory, a man from Canton with black hair and a small pair of hands, thanks to which I was able to mount crystals into the capillaries quickly and accurately, and this impressed Dorothy.'

Last May, Liang was told by Guy Dodson that Dorothy wished to come to China this summer for the 16th International Crystallography Congress. We were delighted at this news and Liang made a great deal of effort to bring her to the meeting in China. Nevertheless, considering her age, we were a little concerned about her health during the long journey from Oxford to Beijing. Furthermore, the big gathering of crystallographers from all over the world would no doubt give her a very busy and exhausting time. How could we fail to understand her? She would go not only to Beijing but even to the ends of the earth. Her age, the hot weather, the long journey, all these are nothing compared to her science. Science is her life! We could not wait to see her again, in China, for the eighth time.

In the 1950s, many Western people knew very little of China, and even less about Chinese science. Dorothy first came to China early in 1959 with her husband, Thomas Lionel Hodgkin, cherishing her intrinsic sense of duty for human progress and civilization and her enthusiasm for people and science in developing countries. In 1965 she visited China again.

In 1967 a group of young Chinese crystallographers started an X-ray crystal structure analysis of insulin in Beijing, stimulated directly by the successful achievement in the total chemical synthesis of bovine insulin with full biological activity in 1965. This was accomplished by a joint group of Chinese biochemists and organic chemists, and also enlightened by the insulin crystallographic research in Dorothy's laboratory. In 1971 and 1973 we obtained the isomorphous replacement electron density maps of insulin in rhombohedral 2 zinc insulin crystals at 2.5 Å and 1.8 Å respectively. In 1972 Dorothy visited China for the third time, this time bringing with her the Oxford electron density map of insulin, as she knew that the Chinese scientists were determining the three-dimensional structure of insulin. Indeed, we compared in detail our electron density maps, as both maps were drawn at a scale of 1 cm to 1 Å, so there would not be many difficulties in cross-checking them. But, there was actually a moment of acute concern when we first put the maps together and found they were not the same at all! What was the matter? Then it suddenly came to light that one map was upside down compared with the other! This released the tension and everyone breathed again. Later we were happy to find that there was a very good, if not exact, correspondence between the two maps. From then on we always mention 'molecule I Oxford, molecule II in Beijing' when we discussed the insulin dimer structure, because comparison with the Oxford model is unavoidable. During her stay in China, Dorothy went to Shanghai to discuss insulin structure and function relationships with scientists in the Institute of Biochemistry. She continued her travels by flying east to Japan to the 9th International Crystallography Congress. In her lecture there, she kindly spoke of her visit to Beijing and Shanghai, and the results of insulin research in China. This opened to the world the first window of the long isolated Chinese crystallography research. We shall never forget that it was Dorothy, with her noble character and high prestige, who brought the research of the Chinese crystallographers to the international scientific community.

In 1974 the Beijing group reported the detailed crystal structure of insulin at 1.8 Å resolution, and the Shanghai group reported on the interactions of insulin with its receptor in liver and fat cells. These were published in the same issue of *Scientia Sinica*, the leading Chinese journal for natural sciences. Shortly after that we were surprised to read a paper in *Nature* (1975, **255**, 103) by Dorothy Crowfoot Hodgkin entitled 'Chinese work on insulin', which commented on the two papers mentioned above. We could only publish our results in domestic journals at that time, and very little was known of these overseas, even of the leading journal *Scientia Sinica*. At that time, under the shadow of the Cultural Revolution, we had to quote from Marx, Engels and Chairman Mao, even in the scientific papers as 'guiding references'. Earlier work on the total chemical synthesis of insulin was also only to be published in *Scientia Sinica*, which made it difficult for the Western world to know what was happening in science in China. Dorothy introduced the achievements of Chinese scientists to the world at an auspicious time through *Nature*. We realize that Dorothy's belief is truth, Dorothy's life is science, she is always enthusiastic about new findings of truth and appreciates scientific achievement whatever its origin.

In this paper in *Nature* Dorothy evaluated our insulin work as follows: 'The present Peking map at 1.8 Å resolution is the most accurate map available of the insulin electron density defined by experimental, isomorphous phase angles... and may well remain so'. We knew the Oxford group was actually carrying out the calculation at 1.5 Å resolution at that time. Dorothy's modesty always impressed us; when she took her first X-ray picture in 1935 many of us were not even born. She never looks down on any small and young group from the superior position of the Oxford group; on the contrary she always shows her concern, gives scientists like us in developing countries encouragement and discusses matters of common interest with us on an equal footing. She said 'One might not wish, in all cases, to see complete duplication of the X-ray analysis of a protein molecule – so much work is involved. But there are great gains in the present case from having two views of the insulin crystal structure.' She has paid close attention to science in China and made very positive comments on a sequence of scientific publications Chinese scientists achieved since 1961, including the total chemical synthesis of bovine insulin, and on collaborations in different disciplines and laboratories. At the end she suggested: 'It will be splendid if we can some day soon all meet and talk over the very interesting observations that are accumulating, East and West, on the structure and function of insulin.'

Dorothy visited China for the fourth time in 1977 accompanied by Guy Dodson. We occupied most of her time here by asking a lot of questions and discussing insulin structure with her. Her very helpful advice was of great value to us.

Chih-chen, the other one of us, a biochemist, was a young member of the insulin group in 1977, but was very lucky to be asked by Liang to take care of Dorothy and Guy during their stay in Beijing. This gave Chih-chen a special chance to be with Dorothy more than any other Chinese scientist can have. In China, a lady is not first, as in the West, but 'elder is first'. Dorothy is an elder and also a lady, and according to traditions in both the West and the East, she should be double FIRST. Even more, she is a great lady and a Nobel Prize winner. All these made Dorothy so great in Chih-chen's eyes, that it is not an overstatement to describe Chih-chen, when she met Dorothy for the first time, as filled with reverence and awe. Moreover, in 1977, only a few people who had studied English before 1949 could speak good English. Obviously it was not easy for Chih-chen to communicate fluently with Dorothy. In Chinese Chih-chen usually has a ready tongue, but then, in front of Dorothy's 'Oxford English' (which everyone knows in China as the standard and most elegant English) she became tongue-tied. Within a very short time however, her awkwardness was swept away by Dorothy's kindness, elegance and affection, and above all by her tolerance.

One day Dorothy visited her old friend Rewi Alley in a quiet typical Chinese bungalow located close to the commercial centre of Beijing. Alley had worked in China for dozens of years and contributed most of his life to Chinese affairs. Dorothy examined a small piece of antique China with great interest, and could not decline to accept it as generous gift from Alley.

On the other side Chih-chen got a present from Dorothy. It was a small piece of dark coloured pottery from Dorothy's home town, as Dorothy told her. It was a treasure to Chih-chen and she put it in the middle, the most noticeable place of the cabinet for keeping all her treasures. She explained to all her friends where this plain-looking art piece was from, with respect and pride. Every gentleman in our group got a T-shirt with an insulin molecule printed on the back, but nobody has ever seen them wearing it. For us Chinese, treasures are for keeping in a safe place and certainly not for wearing.

Two years later Chih-chen worked in Brandenburg's laboratory in Aachen, on a Humboldt Fellowship. She wrote to Dorothy expressing a wish to visit her, and soon after received an invitation from Dorothy. It came in a small envelope with delicate and beautiful handwriting.

In 1979 the Second International Insulin Symposium was hosted by Brandenburg at the Deutsches Wollforshungsinstitut in Aachen. Three Chinese were present among 180 participants from many countries. The Symposium was a great success and Dorothy's wish expressed in her 1975 *Nature* paper materialized. The Proceedings were dedicated to Hodgkin as the wish of everyone working in the insulin field.

Later, in 1980, Dorothy paid her fifth visit to China, accompanied by the Blundells. At the same time, at Dorothy's suggestion, the Sakabes from Nagoya were also invited to Beijing in order to discuss the insulin structure with the three groups together. It was, indeed, a most helpful discussion. However, what we would like to tell is something else. This was Dorothy's fifth visit to China, but she had never found the time to go to the Great Wall, and we strongly recommended that she should visit that Symbol of China. Climbing the Great Wall is quite strenuous work; some parts are very steep, close to 45 degrees inclination. Dorothy was very excited about the climb and very much enjoyed the splendid view of the Great Wall on the towers of beacon fire. Dorothy was happy to become a Chinese 'haohan' which means 'true man', as we have a Chinese proverb 'One is not a true man if one has never been on the Great Wall'. Sakabe seemed to be a very professional cameraman, as good as with his X-ray camera. Many pictures in Liang's album record that happy time. In 1980 Liang had just returned to Beijing from his re-education by way of the Cultural Revolution in Canton, and could afford to invite Dorothy, the Blundells and the Sakabes to his small apartment to have a cup of Chinese tea. It was a great time for Liang's family, especially for the two children who were very proud to have a picture taken with such great scientists; they looked like two bodyguards standing on either side of Dorothy.

In 1985, when Dorothy visited China for the sixth time accompanied by Guy Dodson, we compared the electron density maps of our two groups once again in Beijing at a high resolution of 1.2 Å in our map and 1.5 Å in that of the Oxford group. The agreement of the two maps was surprisingly perfect. David Phillips commented on the method for expanding and refining the resolution of a protein structure determination in a letter to us later, when he learnt from Dorothy of the perfect agreement of the two maps. These maps were done at high resolution through independent refinement and correction of one protein structure in two laboratories, and indicated that the method can be used reliably, and this provides an impetus to protein crystallography development.

Thanks to Dorothy's help the relationship between Chinese crystallographers and insulin researchers and the international community developed rapidly. We now have close connections with many laboratories through a variety of scientific exchanges. One could often see Chinese in Oxford, London, York, Aachen, Hoechst, Boston, HIH, Eli Lilly, Duarte, Brussels, Tsukuba, etc, and there are also Western insulin people in China.

In the past 25 years starting with the insulin structure at 2.5 Å resolution, we now have a fine structure at 1.2 Å as well as structures of dozens of insulin analogues at different resolutions. We also have structures of other proteins, such as trichoxanthin, D-glyceraldehyde-3-phosphate dehydrogenase, phospholipases and many others. We are currently fairly well equipped to do research on X-ray crystallography structure analysis. A number of graduate students have been trained in our Institute during the past years, and there are now quite a few groups in China engaged in X-ray analysis of biomacromolecules. Dorothy's help and friendship have a rich harvest.

A short time later we were very happy to see Dorothy again in Beijing at the 16th International Crystallography Congress. Now, Ramaseshan kindly sent us a copy of the printed pages before publication. Dorothy has left us for more than one year. The last opportunity we had to arrange her eighth visit to China for the 16th International Crystallography Congress compensated a little our great grief for the loss of her. However, the memory of Dorothy lives forever among the Chinese scientists.

Dorothy Hodgkin, protein crystallography and insulin

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Dorothy Hodgkin, or Crowfoot as she then was, began her research into insulin's crystal structure in 1935, soon after moving to Oxford. The crystals were rhombohedral and easily grown following Abel's recipe published a little earlier. Insulin, being a protein and a hormone, was a natural study for her to begin following the experiments that she and J. D. Bernal had carried out on pepsin crystals and on the analysis of steroids. In these pepsin experiments they had established the techniques for obtaining X-ray diffraction patterns from protein crystals. These relied on placing the wet crystal in a sealed glass capillary with a reservoir of mother liquor. Under these conditions the protein crystal lattice retains its waters of crystallization which typically constitute 50% of the cell volume. This technique is still almost universally used today, and only the recent introduction of the cryoscopic method is likely to replace it in any significant way. With this strikingly simple experimental trick, Dorothy in Oxford was able to carry out preliminary X-ray investigations on a number of proteins and characterized crystals of β lactoglobulin, tobacco seed globulin and of course insulin. Most remarkably she studied crystals of tobacco necrosis virus reporting the results in *Nature* after the war.

The insulin studies were Dorothy's major commitment in protein structural research. Once the problems of mounting the crystal and recording their diffraction patterns on film were resolved, the challenge of determining the structure of protein crystals presented itself. At this time, the 1940s, a few small molecule crystal structures had been solved, some of these by Dorothy herself. The problems faced were truly formidable—not even the exact molecular weight of the hormone was known. The evidence from Svedburg was of a 36,000 molecular weight species which could dissociate to a species 12,000 molecular weight. This observation corresponded nicely to the constitution of the rhombohedral crystals whose unit cell contained the 36,000 Dalton species, and the asymmetric unit the 12,000 Dalton species. Not daunted by the complexities Dorothy went ahead and calculated the Patterson function almost as soon as it was published in 1935 for both the wet crystals and the partially dried crystal, for which data extended to 6 Å spacing. The attraction of this calculation was that it required only experimental observations not phases. The disadvantage was that the function generated the vector peak between each pair of atoms in the cell. For the insulin crystal the complexity of the unit cell made structural interpretation of the resultant maps inconceivable. But the Patterson maps of the wet and dry crystal revealed that the two series exhibited marked similarities, and that their main features were preserved. There were, however, relative movements of these features. These experiments suggested that loss of water and the accompanying loss of crystal quality was associated with rearrangements in the crystal lattice but that this phenomenon did not alter the protein structure significantly. These were important experiments in understanding the properties of proteins; they required incidentally considerable skill and a bit of luck. Not all air dried protein crystals diffract, and it was fortunate that the rhombohedral crystals survived this treatment well enough to yield 6 Å data.

With purified insulin and well-defined recipes from Jorgan Schlichtkrull for a second rhombohedral insulin crystal, which also contained zinc, Dorothy returned to concentrate on the crystal structure in the mid 1950s. This step was stimulated by the determination of the chemical sequence of insulin by Fred Sanger and his colleagues, and by the success of John Kendrew and Max Perutz in preparing heavy atom derivatives in myoglobin and haemoglobin. A research group which over the years got quite large and very international, grew up around the insulin crystallographic research.

There were numerous difficulties presented by these rhombohedral crystals, the most serious being their poor ability to react with heavy atoms and the absence of centric reflections which were very convenient for analysing heavy atoms at that time. Their absence meant the heavy atom scattering in the derivatives was not directly measurable from the isomorphous differences, limiting the Patterson functions and the refinement of heavy atom atomic parameters. These difficulties encouraged other approaches. Probably the most important of these were the Patterson based rotation and translation functions being developed in Cambridge by Michael Rossmann and David Blow. They applied these methods to the two different but related rhombohedral insulin crystals, both of which contained two independent molecules in their asymmetric units.

The solution to the insulin structural analysis problems was to exploit anomalous scattering effects. Their measurement made it possible to construct the heavy atom scattering for all reflections, albeit inaccurately. (The mathematical formulations for the expression of the heavy atom scattering were first devised by Luke Hodgkin, Dorothy's eldest son.) They were applied first with partial success to the insulin data by Dorothy and Marjorie Aitken, later Harding, and Margaret Adams. The visit of Sivaraj Ramaseshan in 1964 led to his developing more powerful ways of utilizing anomalous effects in neutrons and most significantly, synchrotron radiation, which has become a major tool in protein crystallography. It was only when the anomalous scattering differences were measured accurately on the Hilger-Watts 4-circle diffractometer by Vijayan, Tom Blundell and myself, however, that the anomalous differences could be exploited effectively in calculations carried out largely by Eleanor Dodson. These analyses revealed that the pattern of heavy atom substitution was surprisingly complex and they needed both accurate and relatively high resolution data (better than 3 Å spacing) to be resolved.

Throughout the period 1955–1969 when the development of the techniques and technologies seemed so slow, Dorothy remained confident that the insulin structure could be solved. She provided a continuous flow of ideas and suggestions. When the structures of myoglobin and lysozyme were determined, for example, she immediately went to see for herself (taking the laboratory with her) what the molecules looked like and what might be learned from heavy atom experiments.

The structure of the 2 Zn insulin crystal was determined in 1969, the culmination of 34 years of research by Dorothy and many individuals. The interpretation of the electron density map was completed in an intensely busy and absorbing weekend. There were many moments of excitement – one was the confirmation of the disulphide bond arrangements defined in Sanger's experiments. The zinc coordination was readily established; and the hormone was seen to be organized in the hexamer as three well defined dimers, held together by H-bonds in β pleated sheet arrangements.

The three-dimensional structure of the two crystallographically independent insulin monomers in the dimer was very similar, though some significant differences exist. The molecule's architecture and the spatial arrangements of the sidechains deduced from the electron density largely explained the hormone's chemical properties (for which there was a huge literature). It was also possible now to begin the task of relating insulin's three-dimensional structure to its biological history and to its action on the cell. In spite of the complex nature of the evidence, progress was made and a region identified as a possible binding surface to the insulin receptor. The widespread use of insulin in the treatment of diabetes added a medical dimension to the structure which was immediately applied to understanding better the design of therapeutic preparations. And of course the possibility of identifying the molecule's active surface raised the prospect, very distant, of synthesizing drug molecules which could act properly on the insulin receptor and remove the burden of injections.

Studies on the other rhombohedral crystals which were grown in concentrated chloride were continued, reflecting curiosity about the origin of the chemical and the structural differences in the two closely related cells. To Dan Mercola's and Graham Bentley's and everyone's surprise the insulin hexamer in this second rhombohedral form contained marked structural differences – including a new zinc coordination site. It was not obvious then – and it is still not obvious why this structural rearrangement should be generated by chloride ions. The large extent of the conformational change demonstrated the striking flexibility of the molecule and was at the time the most dramatic example of the intrinsic capacity for structural alterations in proteins.

The hope that the knowledge of the three-dimensional structure would give clear indications of the insulin's biologically active surface were only partially realized. From the very beginning we and many in other laboratories thought about how the binding surface of the hormone might be identified. The evidence was often suggestive but never conclusive, and some of our ideas were wrong. The essential framework for the developments in thinking was, however, clearly defined in the early structural papers. It turns out, however, from recent research at York and elsewhere that the main problem was failing to appreciate fully enough that the molecule can undergo extensive conformational changes, and that some of these undoubtedly occur at the receptor, and in doing this reveals previously buried surfaces.

In Dorothy's research there was a continuing interest in crystallography and its application to protein crystals. During the heavy atom experiments she actively encouraged Michael Rossmann and David Blow to help with their rotation and translation calculation through which clues to the organization of the molecules in the cell could be obtained. Although no phasing information was obtained from these calculations they helped to demonstrate the value of the methods which are now very widely used. Dorothy, building on her experience with vitamin B_{12}, pursued the exploitation of phase determining methods by anomalous scattering. Her contacts with David Sayre and her interest in solving structures led to their studies with Neil Isaacs on applying direct methods on insulin crystals, and particularly in the later years gave great attention to refinement methods.

The most consistent feature of the insulin research after the solution of the crystal structure was the steady extension of the resolution from 2.8 Å to 1.5 Å spacing. Dorothy always intended to complete the 2 Zn insulin structure as a properly refined molecule. This took some time. It involved developing new methods and endless hours of examining maps. In the end the 2 Zn insulin crystal structure was completed, with all protein atoms identified and a comprehensive and detailed description of the water structure. A good deal of this methodological research was never published in detail, which is a pity, but a reflection of the pressures successful protein crystallographic groups often experience.

Dorothy's influence in insulin research was important. It was of great significance in China where an insulin crystallographic programme was initiated in the mid 1960s following the successful accomplishment of the hormone's chemical synthesis. The research led by Liang Dong-cai, was greatly encouraged by Dorothy, and she had great pleasure comparing the insulin structures determined in Oxford and Beijing in 1971. The subsequent growth of protein crystallography in China owes a great deal to the success of their insulin study and Dorothy's support for it. Other examples of protein crystallographic research being started from the Oxford research are the laboratories of Ted Baker in Palmerston North (New Zealand), Guy and Eleanor Dodson (in York), Tom Blundell (in London) and Vijayan (in India) all of whom worked on insulin with Dorothy at Oxford.

After her retirement in 1977 Dorothy continued to work on insulin, giving lectures and in her research concentrating on the refinement of the 2 Zn insulin crystal with the 1.5 Å spacing data. The approach was strictly crystallographic and the protein atoms and the solvent molecules were analysed and refined as rigorously as possible. This study keeps a standard for protein structure analysis and its completion 50 years after the initial experiments illustrates the persistence Dorothy showed in her research and the immense achievement it represents.

Chemical crystallography – Past, present and future

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WE must not forget that the name of the Oxford laboratory was the Chemical Crystallography Laboratory. I think that may have been one of the reasons I wanted to go there. What I knew about chemistry was based mainly on Pauling's book *The Nature of the Chemical Bond*, and what I knew about crystallography came from the first volume of *The Crystalline State* written by Bragg. A Chemical Crystallography Laboratory seemed like the sort of place where I could combine my interest in these two fields, which was unbounded, with my scanty knowledge.

At that time, remember, fifty years ago, chemists were still arguing and even sometimes quarreling about the molecular structures of simple natural products. A would show that the structure of some compound deduced in B's laboratory was incorrect, and a year later B would turn the tables on A by showing that the revised structure was also wrong. In contrast, the structures determined by X-ray crystallography had a satisfying impression of definiteness about them. Molecules were revealed to correspond to objects of definite size and shape, not just intellectual constructs designed to explain chemical reactivity. There was a price to be paid, of course. In the course of determining the structure of a compound by crystallography, no new chemistry was done, nothing new was learned about the chemical reactivity of the compound in question. It seemed to me then, naive as I was, that as one learned more about the systematics of molecules, it ought to be possible to fill this gap, to deduce chemical properties from molecular structure.

One problem was that, in those days, it took a lot of time and work to determine the structure of even a single relatively simple organic molecule. Also some luck. We knew then practically every organic structure that had ever been determined; who had done it, how it had been solved – there was not much choice, trial and error, or some variant of the heavy-atom method. Two-dimensional Fourier projections, using Beevers–Lipson strips or Robertson boards, were about the limit of computational practicability. Even if one went to the trouble of collecting three-dimensional diffraction data, it was a daunting task to do the necessary calculations. This is why most crystal structures determined in those days had a short cell dimension, short enough so that there was not too much atomic superposition in the corresponding two-dimensional projection.

Chemical crystallography? Ionic structures were reasonably well understood; Pauling's Rules were enough to rationalize them and even to predict unknown structures. In the organic chemistry area there was less to boast about, although there were one or two notable achievements. Through J. M. Robertson's work, for example, the bond lengths in aromatic hydrocarbons could be correlated with simple theoretical models – resonance structures. But for the most part, one had to look optimistically into the future. Considering the complexity of chemical structure and reactivity, a data bank of about fifty structures does not offer much possibility for correlating the two. That was the Past.

I shall speak about the present as it passed me by during the past half century. Thanks to the development of so-called direct methods, made possible by newly developed mathematical models combined with enormous increases in computing power and advances in automated measuring devices, the determination of medium-to-large organic and organometallic crystal structures gradually became more or less routine. This does not mean that the structure of every new compound can be solved automatically but many of them can. In many cases the limiting factor is the availability of crystals. One result has been the accumulation of a vast amount of systematic information about crystal and molecular structure, which has profoundly transformed the whole science of chemistry. I summarize this briefly:

1. Molecular structure. a) Constitution: Influence on synthetic chemistry; liberation from burden of proof of structure via synthesis. b) Configuration: Use of anomalous scattering yields absolute structure. c) Conformation: Information about the balance among various non-bonded interactions was essential for the development of molecular mechanics.
2. Atomic motion in solids. The recognition that the anisotropic 'temperature factors' or displacement parameters (ADPs) determined in single crystal X-ray and neutron diffraction analyses are meaningful. For many years they were widely regarded as junk.
3. Experimental electron density distributions.

In the meantime the main focus of interest for many chemical crystallographers has shifted from the molecular to the intermolecular (supramolecular) level of organization.

4. Crystal packing. The study of weak (non-covalent) intermolecular interactions; atom–atom potentials. Polymorphism. We are still unable to predict with confidence the crystal structure of a compound, given its molecular formula. The structure of minimum potential energy is the stable one at 0 K but not necessarily at room temperature.
5. Solid state chemistry. Photochemical and thermal reactions. Let us remember that the topochemical approach to solid-state chemical reactions, which transformed the subject, was pioneered by the late Gerhard Schmidt, one of Dorothy's most influential students. The study of the influence of 'tailor-made' impurity additives on differential face development has led to insights into crystal growth processes (and to an independent confirmation of the correctness of absolute configurations determined by anomalous dispersion methods!).
6. Structure correlation. The recognition that the changes within a structural fragment in different crystal and molecular environments is often coupled in a characteristic way that gives insight into the chemical behaviour of the fragment. A link between the 'statics' of crystals and the 'dynamics' of reacting chemical systems. Structure–energy relationships.

The influence of all this on 20th century chemistry has been profound – in fact many areas of present day chemistry are unthinkable without the help of modern crystallography. It seems ironic that this progress, almost unimaginable 50 years ago, has been accompanied by the virtual disappearance of crystallographic research in many chemistry departments. Crystal structures are now often, perhaps even mostly, determined as part of a service, and, while this is generally done with great speed and efficiency, the service crystallographer almost always has too much to do, she has no time to think about the broader implications of her results – or even to check them for possible mistakes and misinterpretations. Disorder effects can still cause problems that are sometimes recognized as such, sometimes not. The world production of single-crystal X-ray analyses now runs at something more than 10,000 structures a year. With the advent of various kinds of area detectors and still more efficient computers, this mass production of essentially unchecked crystallographic data is going to increase still further. In any case, results of most current crystallographic studies remain unpublished or receive only scant mention in chemical journals. Thus a vast amount of metrical information about molecular structure and about intermolecular interactions is being accumulated. Much of this information – of the published part at least – is being collected in computer readable form in the Cambridge Structural Database (CSD). Whatever the original intentions may have been, this has now developed into a scientific instrument for studying the systematics of molecular and supramolecular structure. Nowadays, I can hardly imagine that anybody reads, say *Acta Crystallographica C*, in order to broaden their general knowledge. It is read only by people interested in the details of some particular structure or class of structures, and I even suspect that many articles are doomed never to be read by anybody once they have appeared in print. So nowadays almost the *only* way to find details of particular structures or classes of structures is through the CSD. The same is undoubtedly true also for the Brookhaven Protein Data Bank. It seems essential that these two organizations should be kept running smoothly and efficiently until well into the coming century, otherwise the only people who will be acquainted with any particular crystal structure are the people who solved it – or the people for whom it was solved – and their near neighbours.

There is no time left to say more about the future, and perhaps that is just as well.

Insulin structure and diabetes treatment

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Insulin fibrils, formed by native and by monomeric molecules, have been prepared. When examined by electron microscopy they are indistinguishable. The packing of the monomeric insulin in the crystal lattice suggests how insulin fibres are constructed; the model explains the accelerated fibre formation by monomeric insulins and identifies mutations to probe the mechanism further.

THE discovery of insulin in 1921 by Banting and Best and its subsequent use in controlling diabetes was a major advance in medicine[1]. The molecule itself, naturally enough, was intensively studied. It was the first protein whose self-assembly was characterized in detail[2]. Dorothy Hodgkin's own crystallographic studies had demonstrated that the rhombohedral crystal unit cell contained a species of about 36,000 molecular weight and the asymmetric unit a species one third of this, corresponding nicely to Svedberg's measurements. It was the first protein to be sequenced[3] and its structural determination in 1969 by Dorothy Hodgkin and her colleagues in Oxford was amongst the early successes in protein crystallography[4]. During this time major advances in understanding the hormone's biochemistry and its role in biological processes were elucidated; its precursor proinsulin was discovered[5] and its mechanism of action via a membrane-bound receptor was recognized[6] – these were discoveries of general importance.

The solution of the crystal structure of 2 Zn insulin gave Dorothy Hodgkin the opportunity she had been waiting for some 34 years, to establish the structural basis of the hormone's self-assembly to dimer and to hexamer, and to rationalize the vast literature on its chemical and biological behaviour[7]. It was much more difficult, however, to locate the molecule's active surface which, through its contact with the receptor, stimulated glucose transport and other metabolic pathways in the cell. Nonetheless, review of the known insulin sequences and the knowledge of the pathway of various modified (and later mutated) insulin made it possible to identify, at least in part, the receptor-binding surfaces, providing a framework for considering the receptor interactions and, more speculatively, the synthesis of smaller insulin analogues[7–9].

In diabetes treatment, insulin is injected into the musculature where it typically forms a deposit. It then dissolves and travels to the blood where it circulates and can act on its cellular receptors. Although insulin injections have proved generally remarkably effective in controlling diabetes, there are several requirements for therapeutic preparations of insulin. First, they must be stable to temperature and to agitation; if suitable precautions are not taken the protein will precipitate, forming insoluble fibrils. Secondly, because of these phenomena insulin in therapeutic preparations is normally in the form of zinc-containing hexamers, since in this state it is much more stable and resistant to fibril formation. Unfortunately, the large size of the insulin hexamer prevents its absorption, since the hexamers or microcrystals have first to be broken down to release dimers and then monomers, which are small enough to get through the tissue. This leads to a sluggish response to the changes in the blood sugar levels which follow ingestion of food or glucose (Brange *et al.*[10] and references therein). Possibly the slow and inappropriate insulin response experienced by diabetics relying on the injected hormone is responsible for the deterioration of small capillaries and blood vessels, leading to some of the eye, kidney and other circulatory complications that a proportion of patients develop after long periods of therapy.

Monomeric insulin

It was realized at the time of the solution of insulin's crystal structure that knowledge of its 3-dimensional structure offered the promise of improving the molecule's therapeutic properties. Protein engineering techniques made this hope a practicable possibility and programmes to engineer insulins with altered aggregation properties, but unaltered biological activity, were now feasible. One target was the creation of a monomeric insulin[11]. Monomeric insulins, it was argued, would be rapidly absorbed by the tissues and offered an obvious solution to the hormone's slow release from the injection deposit. The surfaces that govern dimer formation and hexamer formation had been defined from the structure of the zinc-containing hexamer in the 2 Zn insulin crystal. The close nature of the dimer-forming contacts and some of the hexamer-forming contacts suggested their modification could destabilize hexamers and dimers and thus lead to a stable monomeric molecule. Using protein engineering, two successful strategies were followed to develop monomeric insulins. These were undertaken[11,12] in the 1980s first by Novo Nordisk and later by Lilly.

Novo Nordisk used the strategy to mutate residues that lay opposite to negatively charged carboxylate groups, to carboxylate groups. Thus when dimers formed a charge repulsion would be generated destabilizing this structure[11]. Lilly's approach was based on mutation of the B28 proline which removed favourable van der Waals' contacts between the monomers in the dimer, thus greatly reducing the dimer's stability[13]. These approaches have led to the development of monomeric insulins with improved therapeutic properties, now in clinical trials. However, these monomeric insulins are less stable and much more prone to form fibrils.

Insulin fibril formation

Insulin fibres were first characterized by Waugh in the 1940s in his extensive studies on insulin stability and precipitation[14]. The conditions that led to fibril formation were identified; they included low pH, elevated temperature, organic solvents and agitation. Various factors such as their ready preparation, reversibility and the dimensions suggested to Waugh that the fibres were constructed from either monomers or dimers and that the molecule was largely unaltered in the process[14,15]. Although the fibrils have been rather little studied, there is a considerable body of spectroscopic evidence to show that they contain β-sheet – which of course in the globular molecule is a structural feature of the dimer and not of the monomer[16] (Figure 1). The conclusion that the β-sheet originated from the native dimer has however been undermined by the observation that the monomeric insulins can form fibrils, and that the monomeric insulin without its C-terminal residues formed fibrils more rapidly than native insulin[10].

The role of the B chain C-terminal residues in affecting fibril formation is seen from the behaviour of an insulin cross linked by the peptide bond between B29 and A1. This analogue does not form fibrils, but forms dimers and hexamers as readily as native insulin[17]. These

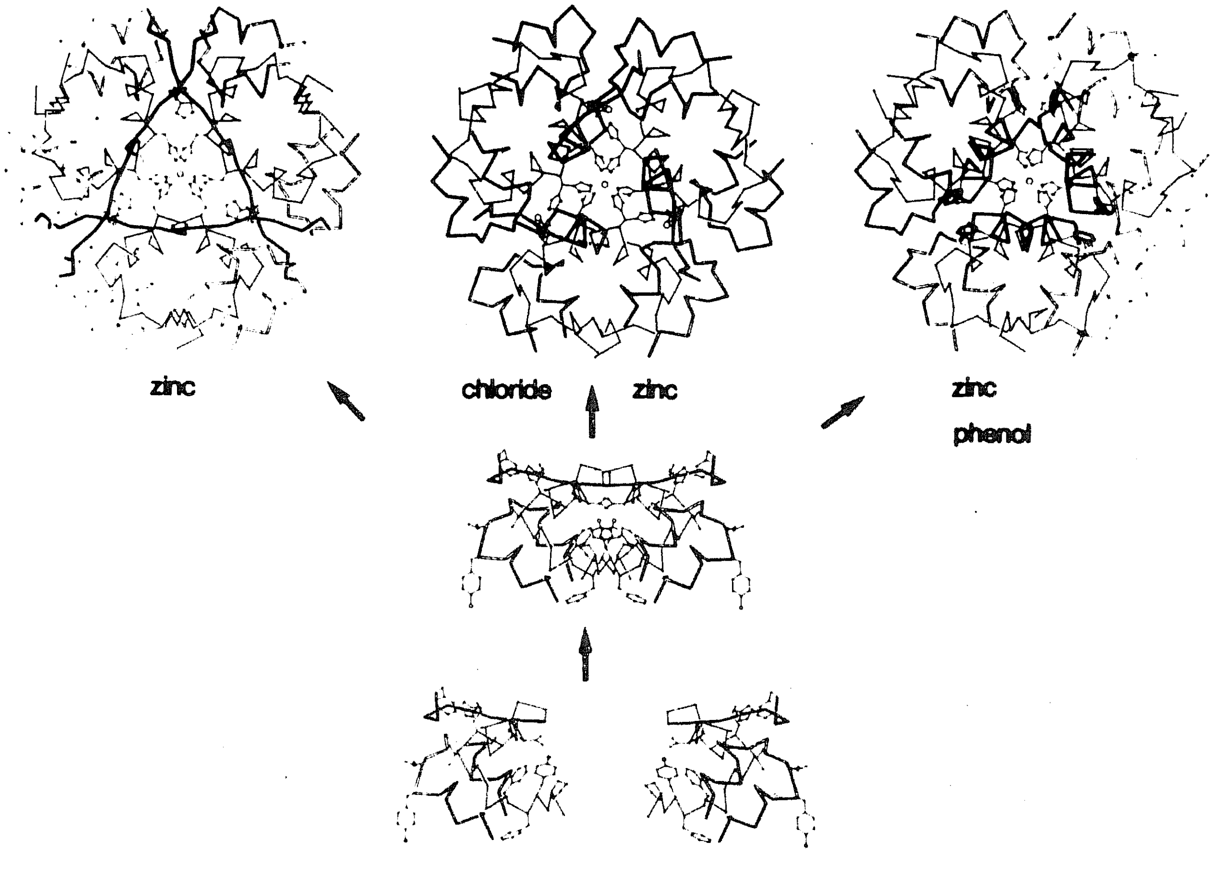

Figure 1. Insulin assembly from the monomer to the dimer and then to the hexamer. The molecules are represented as Cα traces with selected sidechains. The A chain backbone is shown as a double line, the B chain as a thin line. Note that the dimer-forming contacts are almost exclusively made by B chain interactions.

observations suggest that the A1–B29 cross-link prevents movement of the B chain C-terminus that needs to occur for fibril formation. This would explain the ready ability of insulins whose B chain is truncated at the C-terminus (such as des-B30–B26 insulin discussed below) to form fibrils – the B chain C-terminal residues that need to move away are absent.

Crystal structure of monomeric des-pentapeptide (B30–B26) insulin

The monomeric nature of des-pentapeptide insulin (DPI) (which lacks the B-chain C-terminal residues involved in the dimer's β-sheet), its accurate crystal structure and its ready ability to form fibres make it an excellent candidate for investigating the nature of fibre formation.

In contrast to DPI, the native insulin dimer is stable – it is assembled from two monomers, which are packed about a local two-fold axis. The two B chain C-terminal residues form an antiparallel β-sheet with hydrogen bonds connecting residues B24 Phe and B26 Tyr and their two-fold equivalents (Figure 1). In DPI the removal of the residues B30–B26 has little effect on the rest of the molecule but abolishes its ability to form dimers. The near-normal potency of this monomeric insulin[18,19] shows that the B chain C-terminal residues are not important in receptor binding, and are probably displaced during contact with the receptor. This is strikingly reminiscent of insulin and DPI sharing the ability to form fibrils.

The DPI molecule forms unusual crystals for a protein – they contain only 17% solvent by volume[18]. The removal of residues B30–B26 has exposed a good deal of non-polar surface previously covered, and this exposure is increased by the movement of B25 (now C-terminal) away from the molecule. This new surface is largely aliphatic, consisting of A2 isoleucine, A3 valine, B15 leucine together with B12 valine, B24 and B25 phenylalanine, which are on the native molecule surface and are part of the dimer forming surface. These non-polar residues make extensive and important lattice contacts in the DPI crystal with the non-polar sidegroups of the B chain B6, B15, B17 leucines and B14 alanine (Figures 2 *a*, *b*).

It has been proposed that with insulin (and DPI), fibril formation is driven by assembly along these surfaces[20]. Further inspection of the DPI crystal lattice reveals a set of antiparallel β-sheet interactions between the B1 and B4 segments of the two-fold related molecules (see Figure 2 *c*). It is therefore proposed that polymerization of the fibrils through these contacts is responsible for the further polymerization of the fibril and that it

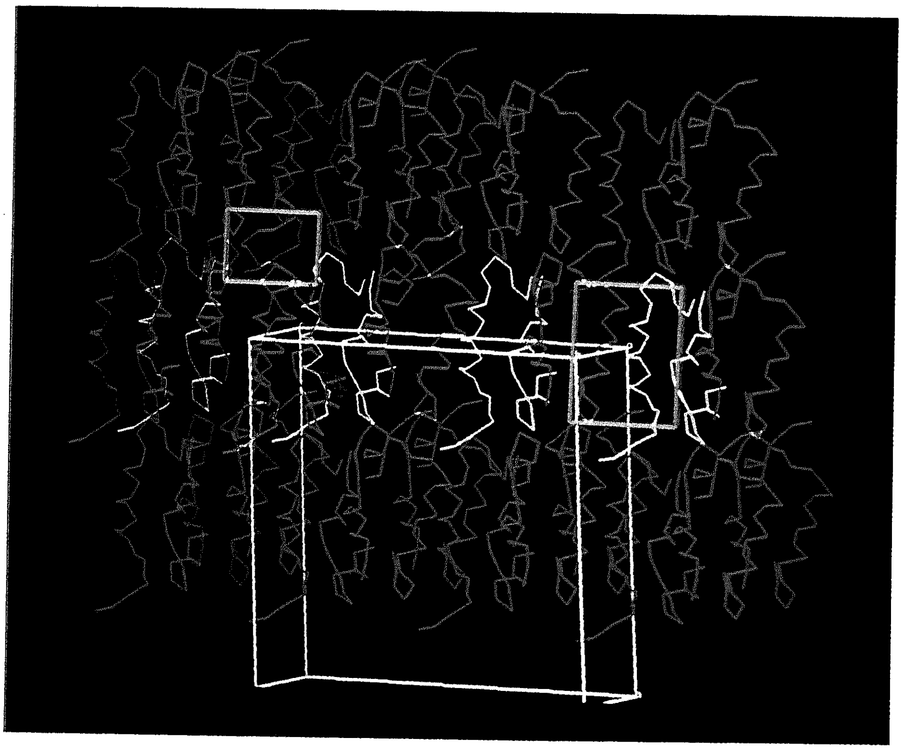

Figure 2 *a*. A schematic view of the DPI crystal packing. The unit cell is outlined in white and the DPI molecules are represented as Cα traces. The red and yellow molecules are linked by non-crystallographic two-fold symmetry axes. Each two-fold related structure is then translated. The two boxes outline respectively Figures 2 *b* and 2 *c*, highlighting the contacts in the crystal.

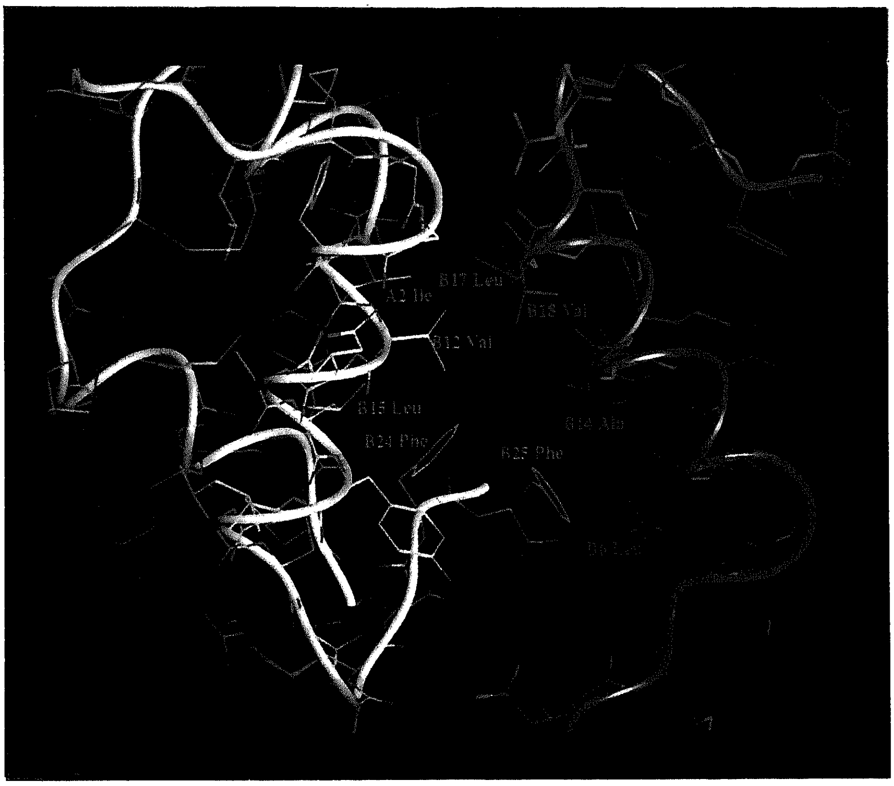

Figure 2*b*. Contacts between two DPI molecules in the crystal. These contacts are non-polar and involve both A chain and B chain residues. This figure distinguishes aggregation between DPI molecules in the crystal from insulin aggregation to the dimer.

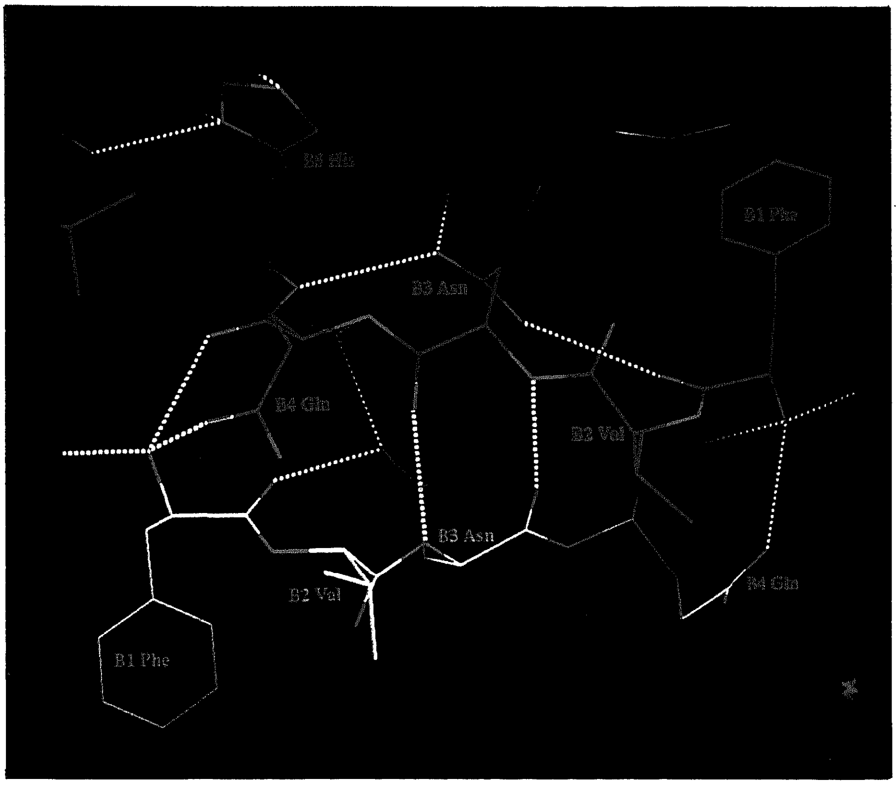

Figure 2*c*. The anti-parallel β-sheet contacts between residues B1 and B4 in the DPI crystal. A local two-fold axis of symmetry exists centred at B3. Note the involvement of B3 and B4 sidechains in important hydrogen bonding contacts.

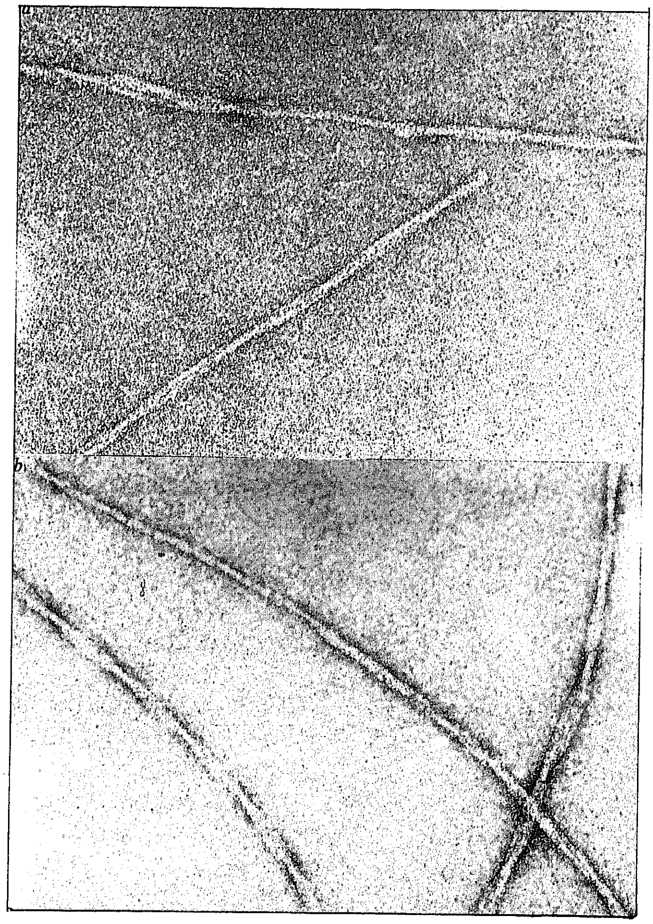

Figure 3. *a*, Insulin fibrils formed with HCl. *b*, DPI fibrils formed with HCl (details in text).

is the presence of this anti-parallel β-sheet structure at B1–B4 that is detected in the insulin fibres. The third surface on the DPI molecule is less likely to aggregate strongly. It includes polar and charged groups and it is largely covered by water structure which helps to mediate the lattice interactions in the crystal. In the conditions of fibril formation tighter interactions between this surface are probable, and may well involve rather different contacts to those seen in the crystal.

Experiments on DPI fibre formation

Fibril formation

In order to compare the fibril characteristics of insulin and DPI, preparations of the fibres were made by two protocols and examined by electron microscopy. The fibril-forming protocols were based on established procedures described originally by Krogh and Hemmingsen[21]; Vigneaud *et al.*[22] and later by Waugh[14].

(i) Samples of native insulin and DPI were made up to millimolar concentration in 1 ml volumes with 0.01 N HCl and heated for 10 min at 100°C and then left to stand. These are referred to as HCl-treated samples.
(ii) Samples of native insulin and DPI were dissolved and then dialysed first against 0.01 N HCl, and then against sulphuric acid (pH = 2). The solution was then heated for 10 min at 100°C and then left to stand. These are referred to as H_2SO_4-treated samples.

The samples were allowed to cool and were left to stand for several days. It was noted that fibril formation, as indicated by viscosity, appeared earlier in the DPI solutions. There were no accurate measurements made on this timing owing to the approximate estimates for determining the state of the solutions.

Electron microscopy

The four samples were examined in the electron microscope after negative staining with uranyl acetate.

Figures 3 and 4 show the electron micrographs of the insulin and DPI samples respectively. It can be seen that both insulin and DPI. when treated only with HCl form fibrils of essentially identical dimensions and character. These appear to consist of two twisted individual fibrils each with dimensions of about 30 Å across. Treatment with H_2SO_4 leads to shorter but stubbier fibres. In these the fibrils have apparently assembled together side-by-side into thicker arrays.

Discussion

The ability of DPI to form fibrils provides conclusive proof that fibril formation proceeds through the monomer and its interactions with other monomers. The fibril forming behaviour of DPI also indicates that a principal mode of contact in fibre formation does not depend on the B-chain C-terminal residues. Indeed the model of the protofibril structure derived from the DPI crystal suggests that it depends on their removal from the contacting surfaces. Analysis of the non-polar contacts in the DPI crystal suggests how these residues would be displaced in the growing fibril without significantly affecting the rest of the insulin molecule's structure[20].

The two-stranded fibre seen in the HCl-treated samples is, we propose, generated by an anti-parallel β-sheet contact between fibrils, leading to the structure described in Figure 2*a*. In H_2SO_4-treated samples, these contacts are more extensive leading to stubbier fibres, an observation which needs explanation.

This pattern of assembly of the insulin fibre from a correctly folded molecule explains naturally many of the characteristics of insulin fibre formation. Thus, the stronger tendency of DPI to form fibres plus the even stronger tendency seen in des-octapeptide insulin (DOI), (Brange, in press[20]) is explained by their monomeric nature, and perhaps by the absence in these molecules of C-terminal residues which would simplify packing along the non-polar surface. The inability of the A1–B29 cross linked insulin to form fibres is equally accounted for by the covalent bond to the B chain C-terminus preventing their displacement. Thus the non-polar contacts that generate the fibril cannot be formed. Finally the dimensions of the protofibril are in cross-section about 30 Å by 50 Å, very much the same as the linear assembly in the DPI crystal (Brange *et al.*, in press[20]).

If the model is correct a number of modifications which would interfere with fibre formation suggest themselves. Thus, substitution of the non-polar residues A13 Leu, B14 Ala and B17 Leu by charged sidechains would impair the non-polar packing made by these residues along the fibre axis. By contrast, removal of the B-chain N-terminal residues should prevent assembly of the fibrils into fibres. The experiments by Waugh and later workers[23–26] have demonstrated that heat, acid pH, agitation and organic solvents all accelerate fibril formation. All these conditions increase monomer formation by mechanical, thermal, chemical and entropic effects, and have been presented as arguments for the fibril process depending on the monomer. By contrast, other factors, such as zinc and calcium ions and phenol, reduce fibre formation. These conditions favour the molecule's assembly to the hexamer, the most stable species in solution, reducing the monomer population and hence fibril formation.

Protein fibres have been a matter for speculation for many years. The early X-ray diffraction studies, electron microscopy and the various spectroscopic analyses that came later, have all been interpreted as indicating the presence of extensive β-sheet structure. A general similarity in the orientation of the β-sheets (the β-strands are perpendicular to the fibre axis) and their characteristic reaction with Congo Red (associated with anti-parallel β-sheet)[27] has led to the proposal that the ultrastructure of amyloidal fibres is similar. Because amyloidal proteins, when globular, have different structures it has been

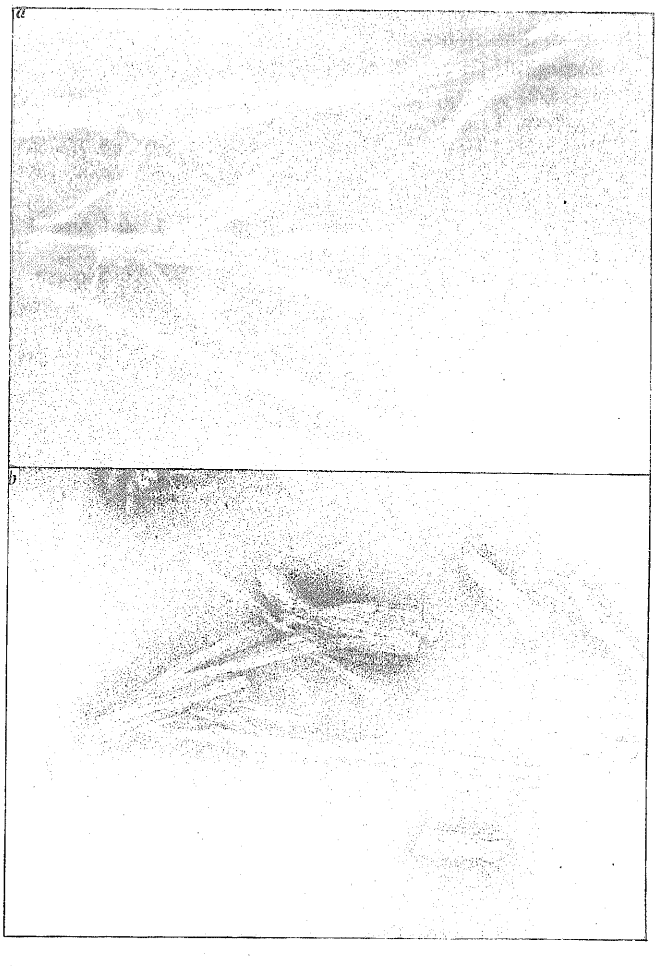

Figure 4. *a*, Insulin fibrils formed with HCl followed by H_2SO_4 treatment. *b*, DPI fibrils formed with HCl followed by H_2SO_4 treatment (details in text).

suggested that the β-sheet structure in these fibres is produced by restructuring of the proteins. By this mechanism the general apparent similarity in their ultrastructure can be explained[28]. It seems from these rather preliminary studies on insulin fibres, however, that they are built from properly folded molecules, which are little altered in conformation by assembly, as Waugh himself proposed[14]. These studies raise the possibility that the apparent common features of amyloid fibres arise from a common, very limited local β-sheet motif formed at the interface of adjacent molecules, as they polymerize. Further research on insulin fibres is essential firstly to establish the structure of their constituent molecules and which features of that structure govern fibre formation, and secondly to relate these findings to the X-ray and spectroscopic evidence.

These studies on insulin fibres were stimulated by the unusual packing of DPI molecules in the crystal. Dorothy Hodgkin would have been delighted but not surprised by the possible correlation between the crystalline state and fibre formation and would have regarded it as the kind of outcome a crystallographer should always be ready for.

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Steroids, steroid-binding proteins and hydrophobic binding sites

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Dorothy Crowfoot's first research assignment was to work on steroid structure, a controversial subject when she started her research. She completed this assignment in 1945 with the first three-dimensional structure of a steroid molecule, cholesteryl iodide. More recently, the structures of proteins which bind steroids have been determined. Some of them have a large closed cavity, big enough for the steroid nucleus, filled with up to 13 molecules of ordered water which must be displaced when a steroid is bound. Even more extensive arrays of ordered water molecules are observed in the binding cavity of some fatty acid-binding proteins. It is suggested that the function of these arrays of ordered water is to enhance the free energy released when a hydrophobic substrate is bound to them. This creates a favourable equilibrium which allows the enzymes to draw in these substrate molecules from their existing hydrophobic environment in micelles or membranes.

In October 1928, Dorothy Crowfoot went up to Somerville College, Oxford to study Chemistry (Figure 1). During her first term in Oxford, two Nobel Prizes for Chemistry were announced, which were to have a close relevance to her subsequent career. No Nobel Prize for Chemistry had been announced in 1927, and in 1928 a prize was awarded for the previous year in addition to the 1928 prize.

The 1927 Nobel Prize for Chemistry went to Heinrich Wieland for 'extracting from bile the saturated acid that is the underlying substance of all bile acids and accurately ascertaining its structure', and the 1928 Prize to Adolf Windaus for his studies on cholesterol (from which he derived cholanic acid) and on ergosterol obtained from milk, which he showed to assume the properties of vitamin D after ultraviolet irradiation.

In Wieland's Nobel lecture, whose readership was extended by republication in *Angewandte Chemie*[1], he summarized the current state of his work and presented a structural formula (Figure 2) which was also in full agreement with Windaus' results. Perhaps he was challenged by the wording of the citation. In Fieser and Fieser's classic book on *Steroids*[2], they write that he 'summarized the conclusions reached with a tentative formula, even though this was incomplete and uncertain. Republication of his Nobel lecture thus gave undue prominence to a curious formula ... that represented merely the working hypothesis of the time'. Although research soon showed it could not be totally correct, this structure became the accepted formula for the steroid nucleus. The central carbon atom participates in three rings, which would give the molecule a rather globular shape.

In 1932, Dorothy Crowfoot, having completed her undergraduate course, moved to Cambridge to begin research under Desmond Bernal. He had been crystallizing several steroids, and earlier that year he triggered a serious controversy when he published the unit cell dimensions of his crystals[3]. He pointed out that these dimensions, especially a 7 Å dimension found in many crystal forms, were 'difficult to reconcile with the usually accepted sterol formula'. Bernal was in touch with the chemists Rosenheim and King at the National Institute for Medical Research in London, who published

Figure 1. Dorothy Crowfoot aged about 20.

new studies suggesting 'that the ring system of the sterols... is that of chrysene, and consists of four six-membered rings'[4], an interpretation which Bernal recognized 'to explain most of the general properties of the crystals whose structures have been studied'[5]. Less than three months later, Wieland's assistant Butenandt, who was to win a Nobel prize himself in 1939 for his work on steroid sex hormones, published a similar suggestion[6]. He proposed 'the existence of four rings' as in 'the new formula recently suggested by Wieland and Windaus' citing 'several papers in press'. He published for the first time the correct formula for the steroid hydrocarbon nucleus without explaining the reason for making ring D five-membered. He infuriated Rosenheim and King by not citing their work, and in almost the next issue of *Nature* they published a stinging letter[7] which pointed out that it was 'not necessary to refer, for "similarities" to the proposed ring system... to papers... which are still "in the press"', since their own publication already presented the proposals. They also stated that their own observation of chrysene formation 'can obviously take place whether the attached ring [D] is six- or five-membered' even though they had specifically suggested four six-membered rings three months earlier. Wieland and Dane's paper[8], which had been submitted before this acerbic exchange had taken place, was published the following month. It presented experiments which gave clear evidence that ring D is five-membered, but was generous in acknowledging the contribution of Rosenheim and King's experiments, which suggested the chrysene-like arrangement of the rings. This evidently calmed the storm, and two months later Rosenheim and King published their own paper[9], reaching the same conclusion, and giving full recognition to the prior work of Wieland and Dane.

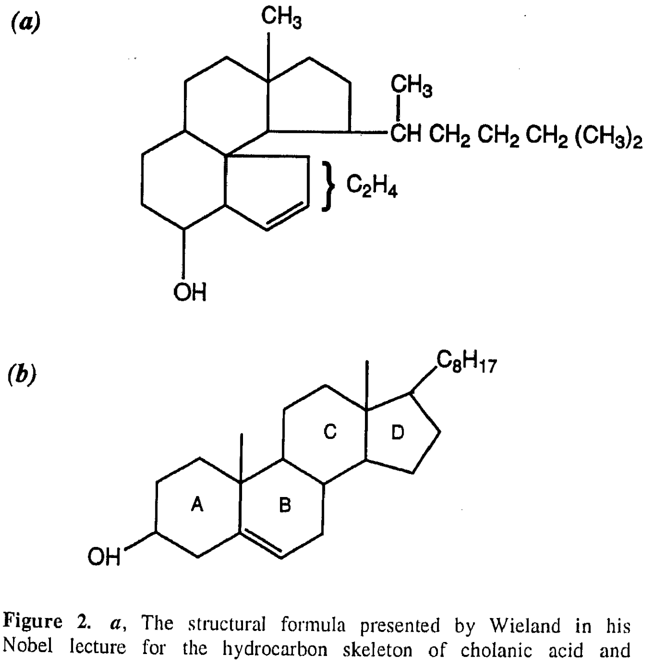

Figure 2. *a*, The structural formula presented by Wieland in his Nobel lecture for the hydrocarbon skeleton of cholanic acid and ergosterol; *b*, The accepted formula of cholesterol.

The rumbles of this storm extended widely, and Dorothy must have been very aware of them. There is a kind of archaeological evidence of the depth of the controversy in text books written decades later. Fieser and Fieser[2] summarized it by a series of dated molecular structure diagrams. Witzmann[10] presents a series of almost identical dated diagrams, which have one tiny difference, giving priority to the German group rather than the British, and in fact this may well be justified. But it seems plausible that Dorothy Crowfoot's early experience of scientific controversy over priority, and bickering over who said exactly what and when, may have been an important factor in her wonderful record of constructive international collaboration and generosity towards competitors.

Three years later Bernal and Crowfoot[11] published crystallographic evidence that the proposed steroid structure was correct. They showed that a substance known as Diel's hydrocarbon, derived by reducing steroids, was identical to a synthetic hydrocarbon, corresponding to the structure now accepted. This work was not easy, since the presence of impurities in their samples had led to crystals of different shapes and different melting points, but the unit cells were identical, and the crystal properties became more similar after further purification. It is interesting that there is no mention whether the intensities of the different orders of diffraction were the same, but we are still many years away from the possibility of 'solving' a molecular structure of this complexity by X-ray diffraction, and all intensity observations would have been made on a Bernal oscillation camera which is difficult to use in a reproducible way.

Detailed structural work on steroids

Dorothy soon undertook ambitious work towards the crystal structure of a steroid, at a time when other known crystal structures were far less complicated. Even those who understand what was involved in calculating a Fourier transform before digital computer programs were available, probably tend to forget just how laborious it was. With Bernal and Fankuchen[12], she showed that the bromine and iodine derivatives of cholesterol are isomorphous, and a two-dimensional Patterson function of the iodide showed a clear iodine–iodine vector. Today one might think of isomorphous replacement but that was not necessary. Carlisle and Crowfoot[13] published their famous paper on the structure of cholesteryl iodide, using what we now call the heavy atom method of phasing. As we read it today, the paper is written in a matter-of-fact style, and gives little indication how far it extended outside the limits of current knowledge. The three-dimensional Fourier transforms used 301 reflections: every point in the electron density map required the summation of 301 trigonometric terms. It does not mention that this is the first application of the heavy-atom method to molecules in a non-centrosymmetric space group, but it does explain how (in the monoclinic system), a single iodine site, forming a centrosymmetric array, generates an electron density map showing the structure and its mirror image simultaneously.

The illustrations to the paper strongly evoke visits to Dorothy's laboratory. There are the vertical wires with cork markers which she always used. In one diagram, the atomic positions of both the true structure and the mirror image are displayed. The paper describes how the molecular structure was separated from the pair of images, using a cardboard triangle, cut to the angle of 108.5°, with the adjacent sides at a length representing 1.54 Å, to identify triplets of carbon atoms belonging to the same image – a simple but powerful tool typical of Dorothy. Carlisle and Crowfoot's structure finally and utterly vindicated the molecular structures which had been defined with such pain in 1932. The double bond in ring B of cholesterol leads to a rather flat conformation for the molecule, which fits easily into the small 7 Å cell dimension which had drawn Bernal's attention to inconsistency with the 1928 proposals.

Steroid-binding proteins

The first structures of steroid-binding proteins were obtained in Mornon's group on the steroid transport protein, uteroglobin[14–16] (Table 1). Insufficient credit has been given to this work, probably because it was instantly clear that the structure had little or nothing to say about specific steroid recognition. The binding site is obvious, a totally enclosed cavity lying on the two-fold axis of the dimer. Since all steroids are a long way from having two-fold symmetry, it was clear that specific steroid binding must use a different strategy. But, in retrospect, this protein has very interesting features, discussed below.

Regrettably, little can be said about the structure of Δ^5-3-ketosteroid isomerase, because it has only been published at 6 Å resolution[17].

Our work on the structure of a cholesterol oxidase from a *Pseudomonas* species produced a structure with several unexpected features[18]. A strong structural relationship with other flavin-binding enzymes was noticeable in the immediate vicinity of flavin, but the rest of the molecule seemed to have no relation to other known structures. The subsequent publication of structure of glucose oxidase[19] revealed a molecule with a close structural relationship to cholesterol oxidase, even though the only detectable sequence homology is in the flavin-binding domain. Sequence comparisons had identified a family of flavoenzymes including the glucose, methanol and choline oxidoreductases (GMC oxidoreductases)[20]. The structural similarities with glucose oxidase thus revealed cholesterol oxidase as a member of the GMC oxidoreductase family[21].

Cholesterol oxidase contains a large closed cavity, suitably shaped to hold the cholesterol ring-system. The cavity surface is largely non-polar, but adjacent to the flavin at one end of the cavity, five polar residues (glutamate, histidine and three aspargines) form a highly polar cap to it, which obviously defines the position of cholesterol's one hydroxyl group (at C3 in ring A).

Table 1. Crystal structures of steroid-binding proteins

Protein	Ligand	Resolution (Å)	Reference
Uteroglobin $C222_1$	–	1.3	14, 15
$P2_1$	–	1.6	16
Δ^5-Ketosteroid isomerase	–	6	17
Cholesterol oxidase	–	1.8	18
	Dehydroisoandrosterone	1.8	21
Fab′ DB3 antibody	–	2.7	22
	Progesterone	2.7	22
	Five other steroids	2.7	23
$3\alpha,20\beta$ Hydroxysteroid dehydrogenase	–	2.6	24
	Carbenoxalone	2.6	24
3α Hydroxysteroid dehydrogenase	–	3.0	25
17β Hydroxysteroid dehydrogenase	–	2.2	26
Cholesterol esterase	–	1.9	27
	Cholesteryl linoleate	1.9	27

These residues are surrounded by highly-ordered water molecules (Figure 3 *a*), which form part of a network of other ordered water molecules, 13 in all, which fill the cavity.

Access to the cavity seems to be provided by three mobile loops which have relatively high temperature factors. These may swing aside when the enzyme docks to a cholesterol-containing membrane, to provide an entrance to the active site with a highly hydrophobic surface, allowing a cholesterol molecule to displace the water from the cavity. This hypothesis was strengthened when the structure of cholesterol oxidase : steroid complex was available. One of the three loops had moved to a slightly different position, and represented the largest movement of the peptide chain on steroid binding[21].

Crystallization of an enzyme–substrate complex raised extreme difficulty, because cholesterol is highly insoluble in water. Another difficulty was that the long aliphatic 'tail' of cholesterol could not be accommodated in the active site, but must hang outside the enzyme, in a way probably not compatible with the crystal packing. In order to achieve a satisfactory level of steroid binding, three effects were exploited. (i) The steroids chosen for study, dehydrisoandrosterone and pregnenalone, have a nucleus identical to that of cholesterol, but lack the long 'tail'. (ii) Detergent micelles were used to solubilize the steroid, and alcohol was introduced into the supernatant, enhancing the steroid's solubility and thus improving the rate of transport from micelles to enzyme sites. (iii) The enzyme was disabled from participating in a full cycle of its action. This was achieved by removal of oxygen from the supernatant, preventing the oxidation of FADH to FAD which normally follows the oxidation of cholesterol. Enzyme–substrate complex appears to have been trapped by this strategy.

The steroids were bound with partial occupancy in the crystals, sufficient to show clearly recognizable electron density for the steroid (Figure 3 *b*). The protein accommodates the cholesterol nucleus with only small movements of a few adjacent side chains, largest in the loop already referred to. All except one of the 13 ordered water molecules is displaced. This last water molecule is at the end of the cavity close to the active site. It is hydrogen-bonded to O3 of the steroid and to two of the residues (His-447 and Asn-485), which form the polar cap adjacent to FAD.

An array of ordered waters in a pocket had already been reported in the two uteroglobin structures[15,16]. The closed pocket lies at the centre of the dimeric molecule, and is similar in size to that observed in cholesterol oxidase. In the two uteroglobin structures, 14 and 6 ordered water molecules respectively can be identified. In $C222_1$ uteroglobin, the 14 water molecules make only four polar interactions with the protein, with threonine and tyrosine side-chains.

The structures of several other steroid-binding proteins are known (Table 1). Some of these are antibodies, whose binding sites bear little resemblance to the struc-

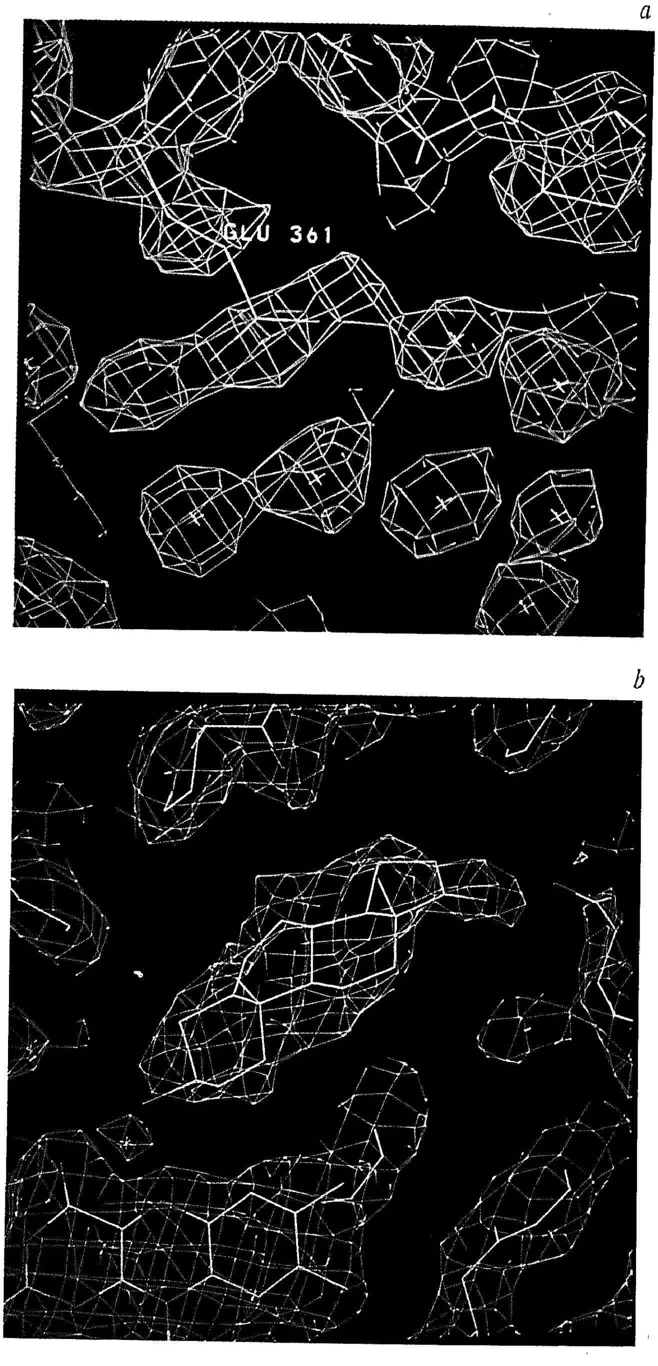

Figure 3. *a*, Part of the electron density in the steroid-binding pocket of cholesterol oxidase, showing 8 of the 13 water molecules. Four water molecules (one at the left-hand edge of this picture) are tightly packed around Glu-361. *b*, Electron density map of the complex with dehydroisoandrosterone, showing the steroid substrate bound in the cavity. The 3-hydroxyl group of the steroid makes a tight polar interaction with Glu-361. It is also tightly bound to the one remaining water molecule in the binding cavity, which appears at the bottom left, adjacent to the flavin. This water is also hydrogen-bonded to His-447 and Asn-485, and possibly to N^5 of the flavin.

tures already discussed[22,23]. In 3α, 20β-hydroxysteroid dehydrogenase, the steroid-binding site is 'deep cleft filled with solvent density' adjacent to a Rossmann fold[24]. Two other hydroxysteroid dehydrogenases have a cavity 11 Å deep at the end of an α/β barrel[25,26]. The latest is cholesterol esterase, a molecule homologous with the lipases, having a spherical hydrophobic cavity which contains some water molecules[27]. None of these publications indicates an extensive network of highly ordered water molecules, though in the structures with resolution worse than about 2.7 Å such a network might be hard to observe.

Other big cavities in proteins which bind hydrophobic substrates

Another class of proteins with large cavities, which bind substrates with predominantly hydrophobic surfaces, is the family which includes retinol-binding proteins, fatty acid-binding proteins and lipid-binding proteins. In at least two of these, both fatty acid-binding proteins, extensive arrays of ordered water have been observed[28–31], especially when substrate is absent (Table 2). So uteroglobin and cholesterol oxidase are not unique in this respect, and the ordered water may have more general significance as a mechanism to increase the affinity of a protein molecules for its hydrophobic target molecules[32].

A wide range of disagreement surrounds the subject of so-called hydrophobic forces[33]. The key problem raised by steroid- and fatty acid-binding proteins is 'How can a soluble protein withdraw a hydrophobic substrate from an environment which already provides a satisfactory hydrophobic-binding surface?'.

It might be thought that the free energy of binding substrate to cholesterol oxidase could easily be found from K_M measurements. This is not so, because satisfactory quantities of cholesterol for assay require its presence under conditions where micelles are formed.

In cholesterol oxidase, the array of ordered waters is maintained by seven interactions with polar groups which are not part of the cavity's hydrophilic cap, which has already been described. They involve a side-chain atom of glutamine and of tyrosine, and four main-chain atoms. When cholesterol is bound, seven hydrogen bonds to

Table 2. Large protein cavities containing water

Protein	Species	Ligand	Bound water molecules	Reference
Steroid-binding proteins				
Uteroglobin	Rabbit C222	–	14	15
	Rabbit P2	–	6	16
Cholesterol oxidase	*Pseudomonas*	–	13	18
		Dehydroisoandrosterone	1	21
Fatty-acid binding proteins (FABP)				
Intestinal FABP	Rat	–	24	28
		14-oate	8	29
		16- and 18-oate	Up to 8	30
Muscle FABP	Human	18-oate	13	31

Table 3. Free energy released on binding steroid

Effect	Assumed unit value	Total energy release
1. Buried ~ 500 Å^2 hydrophobic area *But this is similar to the energy required to remove steroid from a hydrophobic environment in a micelle or a membrane, so in many cases it should be ignored.*	+ 100 J/mol Å^2	+ 50 kJ/mol steroid
2. Lost 7 hydrogen bonds *But several of these hydrogen bonds may be relatively weak because the polar groups on the protein are also making other hydrogen bonds.*	– 6 kJ/mol H-bond	Up to – 42 kJ/mol steroid
3. Disordered 12 water molecules *Depending on how tightly ordered.*	Up to + 10 kJ/mol water	Up to + 120 kJ/mol steroid
4. Ordered one cholesterol molecule *But the cholesterol may also be ordered in the hydrophobic medium from which it is transferred.*	Up to – 180 J/K-mol	Up to – 30 kJ/mol steroid

water are lost. On the other hand, twelve ordered waters become disordered, and a hydrophobic surface of several hundred Å^2 is buried. This disordering of water releases a free energy which may be as large as the latent heat of melting ice, depending on the original degree of order. Using very rough figures, an energy balance sheet can be drawn up (Table 3).

The estimates shown in Table 3 are very rough, and each of them is a high estimate which probably needs adjustment for the reasons given. The energy change on burying hydrophobic area was obtained by Hermann[34] from data on partition coefficients between hydrophobic and aqueous solvents, and corresponds well to the figure established independently by Chothia[35]. The change of free energy on disordering water is obtained from the latent heat of fusion of ice. There is a school of thought which considers that the free energy of burying a hydrophobic surface arises mostly from the water disordering effect. Some may say that items 1 and 3 are different aspects of the same effect, while others consider that there are two separate effects. This emphasizes the vagueness and uncertainty which surrounds the figures.

Table 3 shows two important points. (i) There is ample free energy available to create very tight substrate binding. (ii) The largest term arises from the disordering of water. Even if it is halved to allow for water being partly disordered in the site, it remains the largest term, and it outweighs a high estimate for the compensating energy gain in burying polar groups. It is much larger than in the usual case, because of the highly ordered nature of the water displaced when substrate is bound. This leads to the following suggestion:

> When a highly insoluble molecule such as a steroid binds to a soluble protein, a hydrophobic site in the protein can do little more than balance the energy gain in detaching the steroid from its normal hydrophobic environment. A further loss of energy can be achieved if ordered water is removed from the protein site and transferred to bulk water. Provided the water is sufficiently ordered, this loss is well able to compensate for the abolition of the few hydrogen bonds which are needed to maintain ordered water in the protein site.

Sadly, we were never able to discuss this work in detail with Dorothy Hodgkin. Not only would she have been excited to see cavities shaped to enclose the cholesterol structure which she had discovered. She would undoubtedly have had imaginative and insightful suggestions for further advancement of this work.

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Aspartic proteinases: From the first X-ray photos of pepsin crystals to hundreds of 3-D structures

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Historical background

ALTHOUGH the existence of pepsin in the digestive juices had been noted in the early part of the 18th century and Darwin had identified a similar activity in pitcher plants in 1875, the pepsin family first attracted the attention of biochemists in the 1920s. They were characterized as a group of proteinases, active in the pH range 1–5 and subsequently crystallized in Svedberg's laboratory, leading to the demonstration in the 1930s by Dorothy Crowfoot (Hodgkin) and her supervisor J. D. Bernal that the crystals diffracted X-rays. To quote from Bernal and Crowfoot[1]: 'Four weeks ago, Dr G. Millikan brought us some crystals of pepsin prepared by Dr Philpot in the laboratory of Prof. The Svedberg, Uppsala. They are in the form of perfect hexagonal bipyramids up to 2 mm in length of axial ratio $c/a = 2.3 \pm 0.1$. When examined in their mother liquor, they appear moderately birefringent and positively uniaxial, showing a good interference figure. On exposure to air, however, the birefringence rapidly diminishes. X-ray photographs taken of the crystals in the usual way showed nothing but a vague blackening. This indicates complete alteration of the crystal and explains why previous workers have obtained negative results with proteins, so far as crystalline pattern is concerned.' They go on to comment that, 'At this stage, such ideas are merely speculative, but now that a crystalline protein has been made to give X-ray photographs, it is clear that we have the means of checking them and, by examining the structure of all crystalline proteins, arriving at far more detailed conclusions about protein structure than previous physical or chemical methods have been able to give.' These observations set the scene for modern protein crystallography and enzymology.

Unfortunately, the hexagonal crystals (Figure 1) diffracted weakly and the *c*-axis of the unit cell was more than 300 Å in length, making X-ray data collection difficult in the early days of protein crystallography. But the influence of Dorothy Hodgkin was felt in other ways. The X-ray crystal structures of three fungal aspartic proteinases were each solved by a former member of the Hodgkin/Powell Laboratory of Chemical Crystallography, Oxford University. Thus, Mike James, a student of Dorothy Hodgkin in the 1960s, solved the penicillopepsin structure in Edmonton, Canada[2]; David Davies, who was a student in Oxford with H. M. Powell, but sharing a laboratory with Dorothy Hodgkin in the 1950s, solved rhizopuspepsin in Washington, USA[3–5], and Tom Blundell, who did his post-doctoral work with Dorothy Hodgkin in the late 1960s, solved the structure of endothiapepsin in Sussex, UK[3,6], the latter two structures being first published together.

Dorothy Hodgkin made other indirect contributions to the work on pepsins. When the method of isomorphous replacement proved to be difficult for chymosin and endothiapepsin, Dorothy provided much encouragement. Charles Bunn and his team at the Royal Institution were frequent visitors to Oxford in the 1960s, and on his retirement he passed these projects to the laboratory of Tom Blundell, where they were solved in the following years. The hexagonal pepsin (Figure 2 *b*) itself proved quite a difficult analysis and was not solved until many years later in the Blundell laboratory at Birkbeck[7], sometime after the structure of monoclinic pepsin (Figure

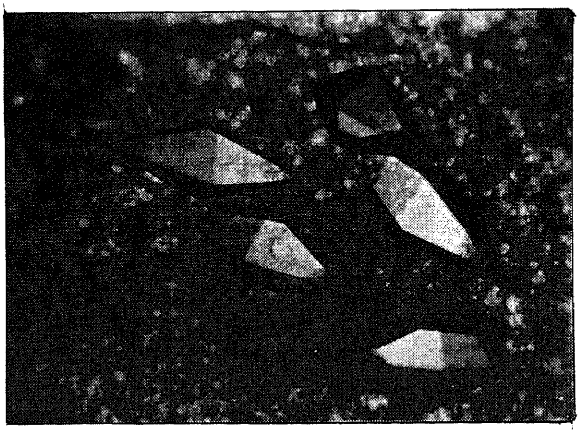

Figure 1. Hexagonal crystals of pepsin.

2 *a*) had been solved in the Andreeva laboratory in Moscow[8].

Many laboratories are now involved in structure analyses of aspartic proteinases; three-dimensional structures for about fifteen different enzymes and hundreds of inhibitor complexes, for example, Figure 2 *c* and *d* and Figure 3, and site-directed mutants have allowed us to make good progress in defining binding subsites and suggesting transition states and intermediates of the catalytic mechanism.

Three-dimensional structures

Pepsin itself and the closely related calf chymosin[9,10] are bilobal enzymes with rather open active site clefts, consistent with binding a wide range of amino acid sequences. More recently the three-dimensional structures of several other enzymes, some of which have more specific roles, have been reported. These include human renin[11-14], the most specific enzyme of the group, which is involved in the formation of angiotensin II, a potent pressor and aldosterogenic substance. Crystal structures have been reported for cathepsin D (refs 15, 16), an enzyme implicated in the invasiveness of breast cancer, and proteinase A (ref. 17), a lysosomal proteinase which is found in the vacuoles of *Saccharomyces cerevisiae* and involved in proteolysis during nitrogen starvation and proteolytic maturation of other vacuolar enzymes. These structures have a similar general topology to pepsin, but in addition a further flap partially covers the active site in a way that might contribute to specific interactions with the substrate.

Inhibitor complexes

The first inhibitor complexes to be studied by X-ray analysis were pepstatin with rhizopuspepsin[4,18] and a pepstatin fragment with penicillopepsin[2]; these confirmed that the hydroxyl group of the statine mimicks a tetrahedral intermediate, being hydrogen bonded to both catalytic aspartates and replacing the water bound at this position in the uncomplexed enzymes.

These and later studies[5,7,13-16,19-27] showed that the active site cleft (Figure 2 *c* and *d*) is formed between

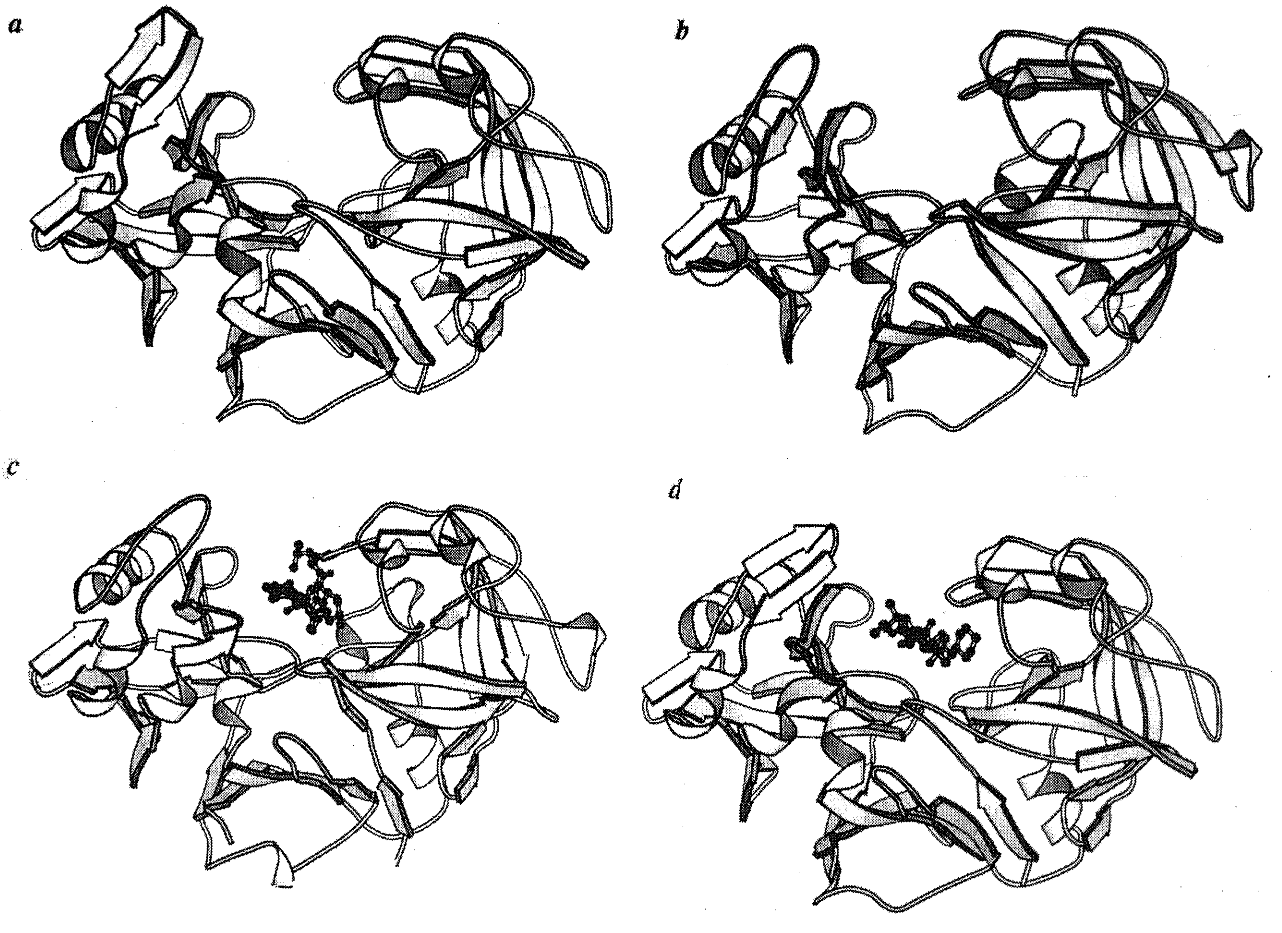

Figure 2. Crystal structures of pepsin: (*a*) 4PEP; monoclinic, (*b*) 5PEP; hexagonal, and pepsin inhibitor complexes: (*c*) 1PSO and (*d*) 1PSA.

the two lobes, each of which provides one of the essential aspartates. The inhibitors have an extended β-strand conformation and make hydrogen bonds through their main chains to each lobe of the enzyme (Figure 3). The side chains alternate on either side of the main chain and make hydrogen bonds and van der Waals interactions with both domains in the molecule, so defining subsites, S6 to S3′. An antiparallel β-sheet, comprising residues 71 to 82 and known as the 'flap', interacts with the central region of the inhibitor and shields the active site from the solvent region. The carboxylates of the active site aspartates form hydrogen bonds to the hydroxyl oxygen of the transition-state isostere (Figure 3). Comparisons of the many inhibitor complexes show that the general mode of binding is conserved over very great evolutionary distances.

In modelling exercises, it was often assumed that specificities derive from differences in the sizes of the residues in the specificity pockets, which complement the corresponding side chains in the substrate/inhibitor[28,29]. This was correct for the S1′ subsites for the human and mouse renins where differences in the enzymes are complementary to the valine and leucine at P1′ in human and mouse angiotensinogens; residue 213 is leucine in human renin and valine in mouse renin. However, the three-dimensional structures of human renin, complexed with a P4 to P1′ norstatine-containing inhibitor CP-85,339 and of mouse renin, complexed with a decapeptide hydroxyethylene isostere inhibitor CH-66 (refs 13, 14), show that the situation can be more complex. For example, the S1′ pockets of chymosin, pepsin and endothiapepsin have an aromatic side-chain at residue 189, while the renins have amino acids with smaller side-chains (valine in human renin and serine in mouse renin). This would be expected to make the pocket larger in renins. However, the structure of the mouse renin complex shows that the substrate moves closer to the enzyme in renins as a result of the smaller residue at 189 and the pocket is made even more compact due to changes in the position and composition of the poly-proline loop (residues 290–297). Thus, the specificity difference at this site arises not only from the movement of a secondary structure, in this case a loop region, but also from the substitution of a residue that allows the substrate to come closer to the body of the enzyme.

The specificity pockets are also influenced by the elaboration of loops on the periphery of the binding cleft in renins. This is most marked at S3′ and S4′. For instance, in endothiapepsin, which has been the

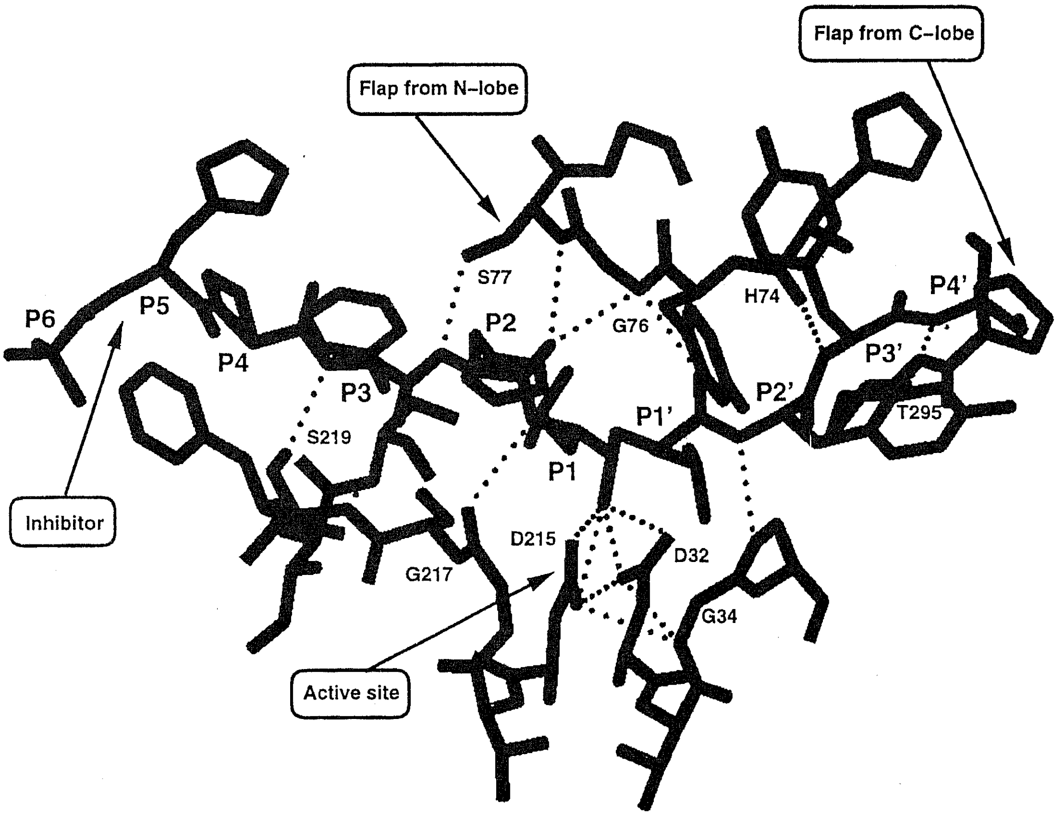

Figure 3. Active site of the mouse renin–inhibitor complex.

subject of the greatest number of studies, different conformations are adopted at P3′, and the residue at P4′ is generally disordered. In contrast these residues are clearly defined in mouse renin. This is mainly a consequence of the poly-proline loop, involving residues 294 to 297. The X-ray analysis of the mouse renin complex shows that the S3′ & S4′ subsites are formed by the poly-proline loop together with residues of the 'flap' and a similar situation is likely to occur in human renin. The well-defined interactions of P3′ described in the mouse renin complex explain the significant affinity when inhibitors have phenylalanine or tyrosine at P3′ as well as the importance of a P3′ residue for catalytic cleavage of a substrate by renin. In human and bovine cathepsin D and in yeast proteinase, this proline-rich loop has a similar conformation but the fact that it occupies a position further away from the inhibitors probably accounts for their decreased specificity for substrates at P3′ & P4′.

Transition-state isosteres and the catalytic mechanism

Although there is no general agreement about the details of the catalytic mechanism, it is clearly established that there is no covalent intermediate involving the catalytic aspartates. It is generally accepted that the nucleophilic attack by a water molecule leads to tetrahedral intermediates and/or transition states involving groups such as $-C(OH)O^{-}-NH_2^{+}-$. There have been several attempts to simulate such tetrahedral intermediates. These have included phosphostatine ($-PO(OH)-CH_2-$), crystal structures of complexes of which demonstrate that the second oxygen binds close to the outer oxygen of Asp-32 (ref. 30). However, this is probably not a very good model as there is likely to be one less proton in the complex than in the real intermediates/transition states. A better model is the hydrated difluorostatone. Thus in the complex of endothiapepsin with the potent difluorostatone-containing tripeptide renin inhibitor (CP-81,282), the scissile bond surrogate is fully hydrated with the pro-(R) hydroxyl oxygen of the inhibitor within 3.4 Å of all four carboxyl oxygens of the two active site aspartates[31].

Two possible complexes with differing locations of the negative charge can be envisaged. In the first, a negatively charged Asp-32 is stabilized by four hydrogen bonds, two from the hydrate hydroxyls, one from Ser-35 γ-OH and one from Gly-34 NH. The outer oxygen of protonated Asp-215 donates a hydrogen bond to the statine-like hydroxyl. In the second, a negatively charged Asp-215 is stabilized by three hydrogen bonds, one from the statine-like hydroxyl of the hydrate, one from Thr-218 γ-OH and one from Gly-217 NH, while the inner oxygen of protonated Asp-32 donates a hydrogen to the statine-like hydroxyl and the outer hydroxyl of the hydrate donates a further hydrogen bond to the outer oxygen of Asp-32. Thus, the high resolution structures of the enzyme inhibitor complexes suggest a mechanism that involves the stabilization of a negatively charged aspartic acid carboxylate first at Asp-215 and then at Asp-32. In each case the stabilization involves complete inaccessibility of the carboxylate from bulk solvent and the formation of three or four hydrogen bonds to the two carboxylate oxygens. This is similar to the environment of several other carboxylates that are conserved in pepsins and which contribute to the negative overall charge at low pH. The absence of any strong stabilization of a developing oxyanion at the peptide carbonyl argues against a possible analogy with serine proteinases, in which main chain nitrogen atoms are thought to stabilize the negative charge of an oxyanion. In the case of the aspartic proteinases the anion stabilized by the enzyme is the negatively charged carboxylate of an aspartic acid, and the intermediate is a gem diol.

Retroviral proteinases

The discovery of pseudo two-fold symmetry in the 3-D structures of the pepsin-like aspartic proteinases by Tang *et al.*[32] has led to a productive series of hypotheses in subsequent years. These hypotheses concern a dimeric ancestor of the aspartic proteinases[32], the close equivalence of the two active site aspartates[33], the similarity of the specificity sites on either side of the scissile bond[28], and the structure of the retroviral proteinases as dimeric homologues of the pepsins[34,35]. The crystal structures of the RSV[36] and HIV proteinases[37,38] have generally confirmed these ideas.

Figure 4 shows representative 3-D structures of monomeric pepsin-like and dimeric retroviral proteinases viewed from equivalent directions perpendicular to the 2-fold axis. The similarity in general shape can be seen although the monomeric pepsin is roughly 60% larger than the retroviral dimer. The close relationship between their folds is best seen by considering the arrangements of the β-strands and α-helices. Each subunit of the retroviral proteinase and each lobe of a pepsin-like proteinase comprises two similar motifs formed from anti-parallel strands: *a*, *b*, *c* and *d* for the first and *a′*, *b′*, *c′* and *d′* for the second[6,36,38,39], organized together in a distorted sheet (sheet 1). Strands *c* and *d′* and strands *c′* and *d* form two pairs of parallel strands. Strands *b* and *c* and strands *b′* and *c′* form anti-parallel β-hairpins that are folded over sheet 1 and hydrogen-bonded together around intra-domain two-fold axis to give a second sheet (sheet 2), which is orthogonal to the first. In the retroviral proteinases the motifs are more symmetrical and less distorted than in the pepsin-like enzymes, where both sheets are fragmented.

Much of strand *a* of the first motif is displaced from the main sheet (1) and forms an anti-parallel β-sheet with the carboxy-terminal strands of the subunit or lobe,

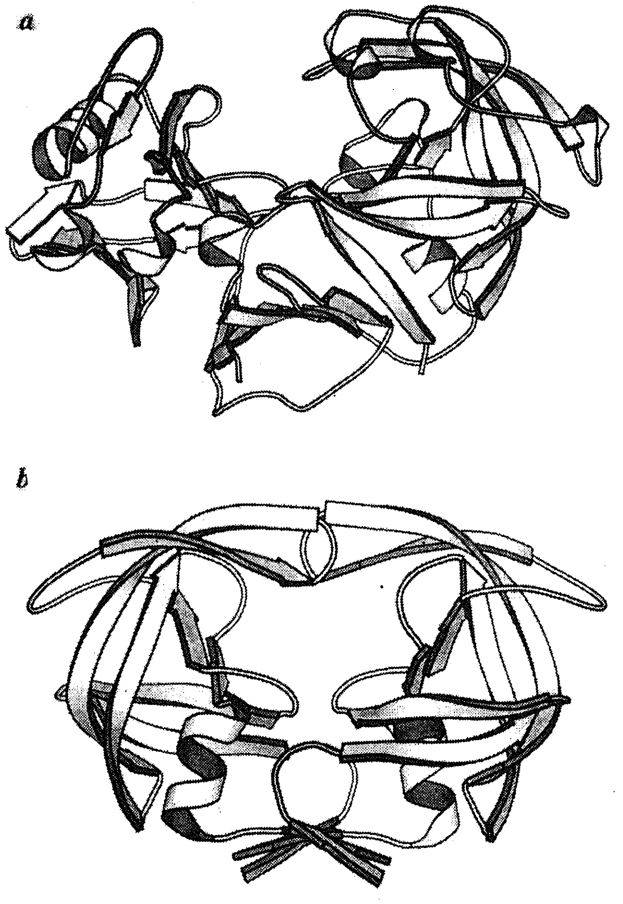

Figure 4. A schematic view perpendicular to the two-fold axis and along the active site cleft of a pepsin-like (*a*) aspartic proteinase[6] and (*b*) the HIV-1 proteinase[37,39].

and their equivalents in the second subunit or lobe (sheet 3). In the retroviral proteinases there is only one carboxy-terminal strand and so the inter-subunit β-sheet contains four antiparallel strands. In the pepsin-like proteinases two carboxy-terminal strands of each lobe contribute to a six stranded antiparallel β-sheet. Figure 4 shows that the strands of β-sheet 3 form the base of the enzymes below the well-defined cleft. In the retroviral and pepsin-like enzymes the equivalent β-sheets occupy the same volume but have different orientations.

The conserved active site residues on the loop between strands *c* and *d*, for example, Asp-25–Thr-26–Gly-27 of the HIV proteinase, form a symmetrical and highly hydrogen-bonded arrangement, virtually identical to that in pepsin-like aspartic proteinases (residues Asp-32–Thr-33–Gly-34 and Asp-215–Thr-216–Gly-217 of pepsin). This includes the two threonines, which are inaccessible to solvent and hydrogen-bonded so that the γ-O forms hydrogen bonds with the mainchain NH and CO functions of the other subunit or lobe in a fireman's grip[33]. They provide a good example of high conservation of buried, hydrogen-bonded polar residues that are important for maintenance of 3-D structure. The two aspartates lie approximately planar with their inner carboxylate oxygens hydrogen bonded to the NH functions of Gly-27 and within hydrogen-bonding distance of each other. The conservation of the glycines appears to be a consequence of the fact that the existence of sidechains at this position would disrupt the structure of the aspartic acid sidechains.

A further significant conservation in the retroviral and pepsin-like aspartic proteinases is the sequence hydrophobic–hydrophobic–glycine found on strand *d′*, for example Ile-84–Ile-85–Gly-86 found in HIV-1 proteinase, which crosses a loop containing an active site aspartate and forms a ψ structure. The equivalents of Ile-85 in other retroviral proteinases and in pepsin-like proteinases are conserved as hydrophobic because they contribute to the core. Gly-86 is packed close to the active site residues in a way that does not allow a sidechain. Ile-84 is conserved for purposes of binding substrate. This pattern is characteristic of both monomeric and dimeric aspartic proteinases. In all retroviral proteinases the following residue is an arginine. Such a basic residue is never found in the pepsins, where it is either a hydrophobic residue or an aspartic acid. In both HIV-1 proteinase and RSV-proteinase[36] this conserved arginine participates in an intersubunit cluster of ionic and hydrogen bonding interactions, and is therefore probably important in dimer formation.

Pepsins in biotechnology and drug discovery

Aspartic proteinases have turned out to have fascinating applications. They have been traditionally used in cheese, soya and cocoa processing and recombinant enzymes are in commercial use in the cheese industry.

Renin is the most specific aspartic proteinase yet discovered and is involved in the formation of angiotensin II, a potent pressor and aldosterogenic substance controlling vascular tone, fluid volume and sodium excretion. Because of its unique specificity, its inhibition was widely expected to provide selective therapy for hypertension, congestive heart failure and associated degenerative disorders linked to angiotensin II. Useful renin inhibitors have been developed by several companies in the 1980s. In the absence of crystal structures most of the early work was based on models of renin[28,29]. Crystal structures of human renin were much later reported at medium resolutions both for the uncomplexed enzyme[11,12] and for various complexed enzymes with inhibitors[12,13]. A higher resolution structure of a mouse renin complex with a decapeptide inhibitor is also reported[13,14]. The detailed analyses of inhibitor complexes indicate the general structural features that may contribute to specificity, especially of renin but demonstrate the need for careful, high resolution X-ray analyses for more confidence in drug design. In particular, they show that even minor alterations in the positions of secondary structural elements can lead to major changes in the disposition of the subsites (see above). Considerable progress was made exploiting structure-based approaches in developing highly specific and orally active molecules. However, most activity in the synthesis of renin inhibitors was stopped when it became apparent that the sophisticated chemistry would require a high price and that they would consequently not be competitive with that of generic antihypertensives, based on the inhibition of angiotensin converting enzyme, as patent protection of these falls. The same technology offers possibilites for anticancer (cathepsin D and E), antimalarial and antifungal agents against candidapepsins, the latter of which can be used to treat infections associated with AIDS.

Most exciting of all has been the production of antivirals targeted at HIV proteinase, which are now proving their value in the clinic. This has been one of the first cases where structure-based approaches have convincingly played a role in the discovery of drugs in the clinic[40]. Structures of several hundred inhibitor complexes have been experimentally defined, providing a previously unparalleled structural database for design. These have exploited a range of different structural features (see Whittle and Blundell[41] for a review) including two-fold symmetry in the ligand, replacement of a bound water molecule, cyclization and replacement of scissile peptide bonds; see for example the cyclic, symmetric inhibitor of the Dupont-Merck team[42]. Several studies have used programs like DOCK or fragment searching to identify non-peptidic structures. Useful molecules are now exploited as cocktails in the treatment of AIDS, with encouraging results, although it is evident that mutation in HIV allows the virus to quickly escape if challenged with a single antiviral.

Conclusions

The structural studies of the pepsins, begun by J. D. Bernal and Dorothy Crowfoot in the 1930s, have had an enormous impact on structural biology, enzymology, biotechnology and drug discovery. Bernal and Crowfoot immediately saw the potential importance for X-ray crystallography in their early studies of pepsin crystals and this has been more than justified. They were also aware of the implications for enzymology; enzymes must be discrete, well-ordered, globular proteins. They could not have foreseen the fascinating story of protein evolution that has since been unravelled, starting from an ancestral dimer to give bilobal enzymes with broad specificites such as pepsin and the fungal aspartic proteinases or highly specific enzymes with higher optimal pHs such as renins. But they would not have been surprised! Neither would they have been surprised by the great value that the knowledge of their structures would have in drug discovery and biotechnology. Dorothy Hodgkin always kept in close collaboration with the pharmaceutical and biotechnology industries, whether she was working on penicillin, vitamin B_{12} or insulin. She was a firm believer that beautiful science will very often be useful. The studies of the aspartic proteinases give further support to her vision.

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Book Review

The Collected Works of Dorothy Crowfoot Hodgkin. Volumes I, II and III. G. G. Dodson, J. P. Glusker, S. Ramaseshan and K. Venkatesan, eds. Indian Academy of Sciences and Interline Publishing Private Limited, Bangalore, 1994.

Dorothy Crowfoot Hodgkin* is a great scientist and equally great humanist of our times. She is considered to be the greatest structural crystallographer. Her work has profoundly influenced chemistry and structural biology. Her influence on crystallographic research in different parts of the world, through the large number of crystallographers trained with her and through her visits, has been far reaching. She has been deeply involved in the communal activities of scientists in general and crystallographers in particular. Her contributions towards bringing people together and promoting international understanding have been significant. That, however, never prevented her from taking strong positions against oppression and injustice. What endeared Dorothy to her students, colleagues, friends and admirers was not only her science but also her personality. Few have been loved as much as Dorothy has been. She radiated affection and goodwill. What made her so effective in the scientific community and outside is mainly her innate goodness.

A compilation of the papers and articles by a many-splendoured scientist like Dorothy Hodgkin is welcome at any time. The publication of her *Collected Works* in the mid-nineties, when her influence is still vibrant and the memories about her are as yet un-dimmed, is all the more welcome. The *Collected Works*, which has been produced in three volumes, contains her writings spanning more than two thousand pages including a short autobiographical, yet unpublished, account of her early life and career. Furthermore, a common set of articles on her written by her selected colleagues from different parts of the world is given in the beginning of each volume. Volumes I and II also contain introductory articles pertaining to the publications contained in them. Volume I is devoted entirely to papers on insulin, Dorothy's abiding interest throughout her career, while volume II deals with papers on sterols, penicillin and other antibiotics, and vitamin B_{12}. Volume III contains structural papers on other compounds, reviews, historical and biographical writings, general articles and her own short memoirs.

In this review, I have departed to a large extent from the normal practice of dealing with the material volume by volume. The papers published during the formative years of Dorothy's scientific career are discussed first. Her approach to choosing scientific problems and dealing with them, as it emerges from the *Collected Works*, is described next. Then the publications presented in Volumes II, I and III are reviewed, almost in that order.

The first publication of Dorothy Crowfoot is along with H. M. ('Tiny') Powell in the form of a short note in *Nature* in 1932 on the double refraction of thallium di-alkyl halides (Vol. III). This was followed up by the same authors with a detailed paper in 1934 on the crystal structure of dimethyl thallium halides deduced from considerations of space group and unit cell dimensions. These publications resulted from the project work ('Part II') carried out by Dorothy under the supervision of Powell as part of her degree course in chemistry at Oxford. Powell himself was young and new to crystallography and Dorothy was his first student. In most of their subsequent career, they worked in the same laboratory at Oxford (known most of the time as the Laboratory of Chemical Crystallography), headed for a long time by Powell. There is however, no evidence for any further serious influence of Powell on Dorothy's scientific career.

Dorothy's true mentor and the greatest influence on her development as a scientist and public figure, was undoubtedly the legendary J. D. Bernal under whose supervision she obtained her doctorate. Bernal and Crowfoot formed a highly productive combination and they co-authored 10 publications on a variety of problems during 1933–35 (Vols III and II). A couple of them formed the starting point for their subsequent well-known work on cholesterol. They also included the celebrated 1934 *Nature* paper on the X-ray measurements of the crystals of pepsin, the first protein to be so studied, which marked the beginning of protein crystallography. They did not co-author many publications, except for a few on steroids, since then, but Bernal remained a great influence and a source of strength and inspiration in Dorothy's career. Her respect and affection for the 'Sage' clearly comes through in very many of her writings included in the *Collected Works*.

Dorothy returned to Oxford in 1934 to start her independent research career and she remained there ever since. Her first independent publications appeared in 1935. One of them was concerned with the interpretation of Weissenberg photographs (Vol. III) and is perhaps the only paper by her exclusively dealing with methods. As often happens in science, but rarely with Dorothy, she was pipped at the post by Martin J. Buerger who submitted a paper on the same topic to the same journal a little earlier. Dorothy, to her utter disappointment, had to be content with publishing a much truncated version of her original manuscript. Another remarkable and historically very important publication by her in 1935 was her *Nature* paper (Vol. I) on the X-ray measurements on insulin. That marked the beginning of her life-long involvement with the structure of insulin.

Dorothy has solved the structures of a large number of compounds belonging to different chemical families. She kept her eyes and ears open for significant problems, examined most of the interesting ones that came her way and then concentrated on the most important and challenging ones on a long-term basis. Of these, the best known have been cholesterol and related compounds, penicillin and related antibiotics, vitamin B_{12} and related compounds, and insulin. She worked on many systems simultaneously, but her primary concern at any given time appears to have been with one or at best two of these systems. In the second half of the thirties she did a great deal of early pioneering work in protein crystallography, especially on insulin, but the intensity of her work in this area gradually waned, to be revived again to the full only in the sixties. The work on sterols, started along with J. D. Bernal in the thirties, continued into the sixties, the last paper being one in 1963 on a calciferol derivative. The work on penicillin started around 1940 (much was not published in the early stages of the work on account of the war) and the last paper to emerge from the Oxford group on a compound related to this antibiotic was in 1973 after a gap of five years since the publication of the previous paper in the series. The first paper on vitamin B_{12} appeared in 1950 while the last contemporaneous primary publication came out in 1975. The two 1984 papers on neutron diffraction studies, published in the *Proceedings of the Indian Academy of Sciences*, were concerned substantially with work done much earlier. Active work on insulin in Dorothy's laboratory was revived in the sixties and a paper on X-ray measurements on insulin crystals appeared, after a gap of 27 years, in 1966. The logical completion of this work, and indeed the effective end of Dorothy's illustrious scientific career, was marked by a long publication on the 1.5 Å resolution structure of 2 Zn insulin in 1988 in the *Philosophical Transactions of the Royal Society*. Thus, her four major research programmes were pursued in successive, telescoping stages. While pursuing these programmes, she also worked on several other projects such as, for example, those involving ferroverdin and thiostreptone.

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*The maiden name Crowfoot was used in most of the early publications while the later publications appear under the surname Hodgkin, acquired after marriage. Most of the time she is referred to by her first name in this review.

Dorothy's papers on cholesterol, penicillin and vitamin B_{12} amply illustrate the distinctive Hodgkin approach in chemical crystallography and its tremendous impact on chemistry. As mentioned earlier, these papers appear in Vol. II. The introductory material in the volume contains, among other general articles, a retrospective on penicillin by Barbara W. Low and on vitamin B_{12} by Jenny Pickworth Glusker, both former students of Dorothy who worked on the respective problems. To most crystallographers, X-ray analysis involves the determination of the three-dimensional arrangement of atoms in compounds with known chemical structural formula. Not so for Dorothy. She determined the chemical formula itself in addition to the three-dimensional structure exclusively through X-ray analysis. Often, as in the case of vitamin B_{12}, the composition itself was in considerable doubt to start with. Thus, as is often said, Dorothy released organic chemists from the drudgery of structure analysis. In each one of her major programmes, she approached the central problem from different directions. For example, the structure determination of vitamin B_{12} involved the X-ray analysis of several of its derivatives and degradation products. Another characteristic of her approach has been the willingness and the ability to use the latest developments in the field. She was among the first to use the Patterson synthesis in the thirties. She always sought and used the latest machines for computation. For instance, she extensively used the computer facilities at the University of California at Los Angeles for the vitamin B_{12} work through the help of K. N. Trueblood. She was an enthusiastic user of neutron diffraction when it became available. Some interesting neutron diffraction studies were carried out on vitamin B_{12}.

The early papers by Bernal and Crowfoot on sterols and related compounds primarily dealt with preliminary studies on their crystals. They did not involve regular structure analysis as we understand it today. They examined about a 100 crystals very systematically. It is remarkable how very chemically significant results could be obtained from simple, but systematic and intelligent, measurements. These measurements led to a drastic revision of the then prevalent understanding of the chemical structure of sterols and contributed to the development of the correct structural formula. The first detailed analysis of a member of the series was that on cholesteryl iodide carried out by Carlisle and Crowfoot and published in 1945. This analysis firmly established the structure of cholesterol. Since then Dorothy and her colleagues worked out the detailed structures of several sterols and published the results during 1948–63.

Dorothy got interested in the structure of penicillin even as the work on sterols was in progress. The war time efforts to produce large quantities of Fleming's penicillin to protect wounded soldiers from infection, are well known. But less generally known is the brilliant elucidation of the chemical structure of penicillin through X-ray analysis which, among other things, made it possible to synthesize it. Penicillin defied structure elucidation by chemical methods. The β-lactam structure of the antibiotic was established by Dorothy and her colleagues by the middle of the forties through the study of sodium, potassium and rubidium benzylpenicillin. This was among the first studies in which a three dimensional electron density map was calculated, long before it became normal to do so. Unfortunately, this very important piece of work was never published in a proper journal. It was, however, described in detail in an article in the book *Chemistry of Penicillins*, published by Princeton University Press in 1949. The publication of a reprint of this article in the *Collected Works* makes it accessible to a wider range of readers. In subsequent years, Dorothy solved and published important papers on cephalosporin, phenoxymethylpenicillin, ampicillin and a synthetic compound related to penicillins and cephalosporins.

A majority of papers in Vol. II are devoted to vitamin B_{12}, the jewel in the crown. They include primary publications, reviews and transcripts of talks. Almost nothing about its structure was known when Dorothy started working on the vitamin in the late forties. The only worthwhile information that she obtained from chemists soon afterwards is that the molecule contained cobalt. The cobalt ion, located from Patterson maps, was the starting point of the analysis using the heavy atom method. For the large molecule that vitamin B_{12} is, the scattering power of cobalt would be considered by anyone except Dorothy as too small for useful phasing. The challenge becomes all the more formidable when even the structural formula is unknown. In the fifties, Dorothy and her colleagues worked on the wet and dry crystals of vitamin B_{12}, a seleno derivative of the vitamin, a hexacarboxylic acid obtained by the degradation of vitamin B_{12}, a chlorine substituted compound and the B_{12} coenzyme adenosylcobalamin. The structure of the vitamin, compact yet complex, with the novel corrin nucleus, was essentially established by the second half of the fifties. Dorothy went on to work on related vitamin B_{12} compounds for many more years. The work on one such compound, factor V 1A, an investigation in which K. Venkatesan was a key participant, represented the first use of anomalous dispersion in the solution of a large structure.

For sheer intellectual brilliance, there are few crystallographic investigations which can match the work on vitamin B_{12}. The structure of the vitamin is a monument to Dorothy's unparalleled chemical intuition and crystallographic prowess. She made sense out of the weak density in early Fourier maps, which appeared to be noise to most others. As David Phillips writes in one of the introductory essays, 'she

Turns them into shapes, and gives to airy nothing
A local habitation and a name'.

She brought to bear on the problem all the relevant crystallographic approaches and the latest technology, especially for computation. Most of the problems involved in solving large non-centrosymmetric structures were enumerated and the methods to overcome them were developed in the course of the structure determination of vitamin B_{12}. They became very relevant later to the crystallography of biological macromolecules such as proteins.

Despite her remarkable successes with cholesterol, penicillin and vitamin B_{12}, the problem closest to her heart was insulin. Insulin remained with her throughout her career and, quite appropriately, one whole volume (Vol. I) of the *Collected Works* is devoted to her work on insulin. Again, quite appropriately, the insulin papers are introduced through an article by G. G. Dodson who worked with Dorothy for the longest period and whose length of association with insulin crystals was second only to that of Dorothy's. The first phase of insulin work was part of the 'ancient history of protein X-ray analysis' (recounted by her and Riley in a paper reproduced in Vol. III). This history started when she and her mentor J. D. Bernal recorded and examined the X-ray diffraction pattern from the crystals of pepsin in 1934. Then occurred in 1935 what she reckons as probably the most exciting event in her life, the X-ray photography of insulin crystals. During those very early days of protein crystallography, she worked on other proteins as well, but insulin was the protein that she concentrated on. That was a time when even the exact chemical nature of proteins was unknown. Yet, along with other pioneers like Max Perutz, she persevered. She recorded X-ray diffraction photographs using a primitive oscillation camera, from dry and wet insulin crystals, indexed them and measured the intensities by eye. Then she did what best anyone could do at that time: calculated Patterson maps, a formidable undertaking in pre-computer days, and looked for general patterns. This work was reported in two papers, one in 1938 by herself and the other in 1939 by herself and her student Riley. That is where matters rested for a couple of decades.

Dorothy's next publications on insulin were in 1966, after a gap of 27 years. Much had happened in the meantime. She had solved the structures of cholesterol, penicillin and vitamin B_{12} and earned the fellowship of the Royal Society and the Nobel Prize. Max Perutz and John Kendrew and their colleagues had solved the structures of haemoglobin and myoglobin by the turn of the decade. The crystal structure of an enzyme, lysozyme, had been determined by the group of David Phillips, by then. The structures of several other proteins were well on the way to solution. Thus, protein crystallography, though still in its heroic days, was well established by that time. Also, the amino acid sequence of insulin, determined by Fred Sanger in the fifties, was available by the time the two 1966 papers were published. The first of the two dealt with the two rhombohedral forms of insulin (2 Zn insulin containing 2 zinc ions per insulin hexamer and 4 zinc insulin containing 4 zinc ions per insulin hexamer), cubic insulin and monoclinic insulin. The second paper was concerned with the first application, on 2 Zn insulin and 4 Zn insulin, of the rotation and translation functions developed by Rossmann and Blow, functions which were subsequently to become so very important in the molecular replacement method of structure solution. This work established the internal symmetry (approximately 32 or D_3) of the insulin hexamer.

Since then the work on insulin progressed rapidly and its much awaited structure in the 2 Zn insulin form, at 2.8 Å resolution, appeared in the centenary issue of *Nature* in 1969 to the accompaniment of much celebration. Insulin was, of course, the first protein hormone to be X-ray analysed. The structure, not surprisingly, provided a wealth of information on the polypeptide conformation, intramolecular interactions and oligomerization of insulin. Most of the subsequent publications on insulin in the volume are reviews, abstracts, articles in conference proceedings and transcripts of lectures, interspersed with a few primary publications containing significant new information. The relationship of the structure with every aspect of the chemistry, biology and evolution of insulin has been examined in considerable detail in these publications. An important publication in 1976 reports the structure determination of 4 Zn insulin in which three of the insulin monomers in the hexamer have the same conformation as in 2 Zn insulin while in the other three, parts of the polypeptide chains exhibit a different conformation. This formed the basis of the subsequent detailed studies on the R and T states of insulin. The publications in the volume also give evidence to Dorothy's affectionate and generous involvement with the Chinese and the Japanese work on insulin. The last of her publications on insulin, indeed her last major scientific publication, is a very detailed account of the 1.5 Å resolution structure of 2 Zn insulin which appeared in 1988 in the *Philosophical Transactions of the Royal Society*. That is perhaps the most detailed account of any protein structure published so far. That also marked the culmination of Dorothy's loving, and often passionate, involvement with insulin spanning more than half a century.

Volume III of the *Collected Works* is devoted, as indicated earlier, to her X-ray work on other compounds, general crystallographic articles, essays on different topics, historical and biographical sketches, book reviews, general lectures and unfinished autobiographical memoirs. The mixed character of the contents of the volume makes interesting reading, but makes the job of the reviewer difficult. Her reviews in the second half of thirties, forties and early fifties provide authentic accounts of the contemporary state of structural crystallography. Many papers and articles are concerned with the early days of protein crystallography. Then there are accounts of some splendid structure determinations. Interestingly, perhaps the last structures Dorothy determined, reported in 1982, were those of two simple cyclohexane derivatives. Here is a great scientist, with so many magnificient achievements to her credit, finding it worthwhile dealing with a problem simple enough to be assigned to a graduate summer trainee! That in some ways illuminates an aspect of her approach to scientific research. She did great things, but never insisted that she would do only great things. She enjoyed every bit of her work, whether it be on the complex vitamin B_{12} or a simple cyclohexane derivative.

The volume contains some excellent biographical sketches on Kathlene Lonsdale and J. D. Bernal, in addition to an obituary on Dorothy Wrinch. All the three have influenced Dorothy greatly and all of whom she knew intimately. The biographical memoirs on Bernal and Lonsdale, written in Dorothy's inimitable intimate style, are small volumes by themselves. The subjects come alive in them.

The articles on Dorothy by a selected few of her colleagues and friends from different parts of the world are a very valuable feature of these volumes. These articles are written by Jack Dunitz, Max Perutz, David Phillips, B. K. Vainstein, S. Ramaseshan, Dong-cai Liang and Chih-chen Wang and Guy Dodson. They discuss, all in very affectionate and intimate terms, the many facets of Dorothy's life, family and personality, her style as a scientist, the ambience of her Oxford laboratory, their relationship with her, her visits, her influence on the development of science in different parts of the world.

While reviewing a book by Sir Lawrence Bragg, Dorothy wrote (Vol. III. p. 618): 'This book is a joy to read. Over and over again one can almost hear W.L. Bragg talking, as he used to talk when he was with us. . . .' The same could be said about Dorothy's writings. This is particularly true about the unfinished autobiographical memoirs at the end of Vol. III. She writes about her family background, her birth in Cairo, her schooling in England, her stay in Sudan and Jerusalem where her father worked, her fascination for chemistry and archaeology and much else on her early life. Then there are her student days at Sommerville (Oxford) and her introduction to crystallography, all discussed in the historical setting. She writes about her short momentous interlude at Cambridge, which probably set the stage for her illustrious future career, and her work and association with Bernal. She returned to Oxford after a couple of years at Cambridge, to set up her own laboratory and career. In her narrative, as indeed in her life, science, friends and family all become parts of a harmonious whole. She describes how she met and got to know Thomas Hodgkin, her husband, their attempts to set up home, though having jobs at different places, and the birth of Luke, their first son. There are references to their homes at Oxford, 315 Woodstock Road and 20C Bradmore Road, and above all to Crab Mill at Ilmington, landmarks so very familiar to her students, colleagues and friends. There are references to the beginnings of her rheumatism which troubled her throughout her life. Then there are accounts of the beginnings of the Second World War and its unsetting effects. Her early exciting work on protein crystallography, the solution of the structure of cholesteryl iodide and many other interesting investigations were carried out during this period when Dorothy and Thomas were settling down at Oxford along with their son, in a rather unsettled environment. The memoirs conclude with the penicillin story, the vicissitudes during the investigation and how the β-lactam structure was finally arrived at by the end of the war. The memoirs cover only the first 35 years of Dorothy's life and less than 15 years of her illustrious scientific career. One wishes that she remained well enough to complete the story!

The editors and the Academy deserve congratulations for producing these splendid volumes which are sure to be of abiding interest to scientists and those interested in science in general. They would be of particular value to crystallographers, chemists, structural biologists and historians of science. To those of us who have had the good fortune to work closely with Dorothy and to know her intimately, the volumes bring not only enlightenment, but also joy and happy memories.

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Purification and pathogenicity of the cytotoxic factor from the cases of dengue haemorrhagic fever

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Pathogenesis of dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) is not yet clear in spite of extensive efforts. Here we report the discovery of a cytotoxic cytokine (hCF) in the sera of the patients with DHF/DSS which is capable of increasing vascular permeability in mice. The presence of hCF was detected in the sera of the cases of DHF by dot blot test. hCF was purified from the sera of 34 dot blot-positive patients of DHF by ion-exchange chromatography followed by high performance liquid chromatography. The eluted hCF was cytotoxic to normal mouse spleen cells, had a molecular weight of 43 kD and an isoelectric point of pH 4.5–5. It reacted in dot blot and Western blot tests with anti-mouse cytotoxic factor (mCF) and anti-hCF-antisera. The effect of hCF on the capillary permeability and the blood-brain barrier of mice was investigated using leakage of Evans blue dye-bound plasma proteins. It was observed that inoculation of the hCF in mice increased the capillary permeability and damaged the blood-brain barrier in a dose-dependent manner. This is the first evidence of the presence of cytotoxic factor (hCF), a homologue of mCF, in the sera of the cases of DHF/DSS which can cause reversible damage to capillaries.

DENGUE viruses usually cause widespread epidemics of a benign illness, dengue fever (DF), which is characterized by an acute onset of severe headache, myalgia and fever lasting for 3 to 7 days and a maculopapular rash appearing towards the end of the illness. The virus also produces a severe syndrome, dengue haemorrhagic fever/dengue shock syndrome (DHF/DSS) which is characterized by increased vascular permeability, thrombocytopenia, alterations in blood leucocytes, cerebral oedema and abnormal haemostasis. The pathogenesis of the disease is not fully understood in spite of extensive efforts[1-3].

Dengue type 2 virus (DV) induces production of a unique cytokine, cytotoxic factor (mCF) by Thy1.2$^+$, and L3T4-positive T lymphocytes of mouse[4,5]. mCF is a highly potent protein molecule of 43 kD MW on polyacrylamide gel electrophoresis (PAGE) and reacts with anti-mCF-antibody in the Western blot test[6]. A comparison of aminoterminal sequence of 19 amino acids of mCF with other similar cytokines and interleukins (IL), such as tumour necrosis factor-alpha (TNF-α), TNF-β, interferon-gamma (IFN-γ), IL-13, IL-14, IL-17 or any other cytokine, T cell proteases and dengue virus-specific proteins did not show significant homology[6]. Oligonucleotide probes derived from this sequence show the presence of mRNA for mCF by Northern hybridization in the spleen cells of DV-infected mice[7]. cDNA library for mCF has been expressed in *E. coli* and compared with the native CF (U. C. Chaturvedi *et al.*, unpublished data). mCF kills selectively T cells, H-2A negative macrophages, megakaryocytes and acts on cells capable of liberating histamine. The action of CF is via production of nitrite and respiratory burst (formation of peroxynitrite) and is calcium-dependent, inducing influx of calcium in the target cells[4,8-13]. The electron microscopic appearance of mCF-treated mouse spleen cells is similar to the apoptotic cells[14]. Recently, presence of high affinity receptors for CF (CF-R) has been demonstrated on macrophages by using ^{125}I labelled CF (A. Misra and U. C. Chaturvedi, unpublished data).

mCF appears to be pathogenesis-related protein, capable of reproducing all the pathological lesions in mice that are seen in cases of human dengue haemorrhagic fever (DHF) namely increased vascular permeability[15], cerebral oedema[16] and blood leucocyte changes[17]. Active vaccination of mice using mCF as antigen protects them against subsequent challenge with mCF and DV given by peripheral route[18,19]. The present report describes our success in demonstration of human CF (hCF) among 220 patients of DHF, its purification and partial characterization from the sera of 34 cases of DHF and its pathogenicity in mice.

Materials and methods

Patients

The cases of dengue admitted to the Children's Hospital, Rajvithi Road, Bangkok were thoroughly examined clinically and were fully investigated at the Virology Department, US Army Medical Component, AFRIMS, Bangkok. In addition to recording age, sex, the cases were clinically graded[2] as dengue fever (DF) and dengue haemorrhagic fever (DHF) grades 1 to 4. In the laboratory, their blood samples were investigated for immunoglobulin profile, serological profile of antibodies against different flaviviruses and isolation of dengue viruses. On the basis of the laboratory findings, the cases were diagnosed as of primary or secondary dengue infection. The rising antibody titre and the virus isolation studies indicated the type (Types 1–4) of dengue virus infection. Sera collected were stored at – 70°C. For the present study, sera collected during 1993 and 1995 were randomly selected. A total of 220 sera found positive on initial screening by inhibition ELISA were selected for the present study.

Purification of hCF

Ion-exchange chromatographic technique[20] was used for initial purification of hCF from the sera of the patients. Diethylaminoethyl (DEAE)-cellulose was precycled with 0.5 N HCl followed by washing with deionized water to pH 7.0 and then treated twice with 0.5 N NaOH, washed with deionized water to obtain a neutral pH; and finally equilibrated with phosphate buffer (PB) pH 7.2. Serum sample (400 µl) was applied to the column. The elution was performed with a linear gradient from 0 to 0.5 M NaCl dissolved in PB with a flow rate of 500 µl/min. Fractions of 4 ml volume were collected and monitored for their absorption at 280 nm (ref. 20) and for the cytotoxic activity using normal mouse spleen single cell suspension as target[8]. This was followed by runs on Pharmacia Fast protein liquid chromatography (FPLC) system with Mono 'Q' columns, under the above conditions. The individual peaks from the different runs were pooled, extensively dialysed against double distilled water and were dried in a Speed Vac (Savant Instrument Company, New York, NY) to concentrate. The protein was further purified on Pharmacia high performance liquid chromatography (HPLC) using reverse phase C18 column. The elution was performed with a gradient of acetonitrile (30%; containing 0.05% fluoroacetic acid) and water (70%; containing 0.05% trifluoroacetic acid). The fractions were monitored at 260 nm. The peak was collected, dialysed and freeze dried. The protein contents of each peak were determined by the method of Lowry *et al.*[21]. The purity of the hCF was ascertained by electrophoresis on SDS-PAGE followed by silver staining.

Preparation of mouse cytotoxic factor

mCF was prepared from the spleen cells of DV-infected moribund mice as described elsewhere[6]. CF was purified with a Pharmacia low pressure liquid chromatography (LPLC) system using Sephacryl S-200 gel column and then on FPLC[6]. The amount of protein was estimated by the technique of Lowry *et al.*[21]. The purity of mCF was established by SDS-PAGE using silver stain. Normal spleen cell supernatant (NF) was similarly prepared and used as control.

Assay of cytotoxic activity

The cytotoxic activity of preparations was assayed using normal mouse spleen cells as target[8]. Spleen cells were teased out in cold MEM to make a single cell suspension and nucleated viable cells were counted. Briefly 100 µl of the test solution was mixed with 100 µl target cells (2×10^6 cells) in a 96 well perspex plate and kept at 4°C for 1 h. Viability of cells was assayed by trypan blue exclusion test and the percentage of nonviable cells was calculated.

Preparation of CF-specific antisera

Antibody against mCF was prepared in mice by injecting 5 µg mCF emulsified in Freund's complete adjuvant, subcutaneously. The injection was repeated after 15 days using the same dose of mCF emulsified in Freund's incomplete adjuvant (Sigma, USA). The blood was collected on 30th day, sera were separated and stored at – 70°C. Similarly antisera against HPLC purified hCF was also produced. Both the antisera specifically neutralized the cytotoxic activity of mCF/hCF and reacted with it in a Western blot test[6]. Sera obtained from normal mouse were used as control.

Polyacrylamide gel electrophoresis

The cytotoxic peaks obtained by chromatography were electrophoresed on 12.5% polyacrylamide gel at a constant current of 30 mA in a vertical slab gel system (Pharmacia, Sweden). For controls, crude hCF and purified mouse CF preparations were included. Standard molecular weight markers (phosphorylase B-107 kD; bovine serum albumin-76, ovalbumin-52; carbonic anhydrase-41.5; soyabean trypsin inhibitor-30.5; and lysozyme-19.4 kD) were also included in some of the runs. The gels were stained with Coomassie Brilliant Blue R-250 stain or silver stain[6]. The above proteins were also run on SDS-PAGE and stained with silver nitrate. The isoelectric point of purified hCF was studied on Pharmacia Phast gel electrophoresis system using Phast Gel IEF 3-9 medium.

Dot blot test

hCF was detected in the sera of the patients of various grades of dengue illness and the controls which included cases of non-dengue illness and normal healthy individuals. Purified mouse CF was used as positive control. The sera were diluted ten-fold with phosphate buffered saline pH 7.2 (PBS) and 100 µl of it was dot blotted on nitrocellulose paper in Milliblot system (Millipore, USA). The blots were air-dried and blocked overnight at 4°C with 3% bovine serum albumin prepared in 0.1 M Tris HCl pH 7.5. After three washings with PBS containing 0.05% Tween 20 (PBST), the blots were incubated with anti-mCF or anti-hCF-antisera[7] (1 : 500 dilution in PBST) on a rocking platform at room temperature for 1 h. After three washings, it was incubated with anti-mouse IgG coupled with horse radish peroxidase (diluted 1 : 500 in PBST) for 1 h at room temperature. After extensive washing, the blots were developed using diaminobenzidine and hydrogen peroxide. To check the specificity of the reaction, some of the blots were treated with anti-dengue virus or anti-Japanese encephalitis virus antisera or normal mouse sera, etc.

Western blot analysis

The samples resolved on PAGE as above, were electroblotted on nitrocellulose paper. The blots were air-dried and developed as in dot blot test described above. The samples included mouse CF as positive control and a number of negative controls.

Assay of capillary permeability

Measurement of leakage of plasma protein-bound Evans blue dye from the vascular compartment into peritoneal cavity of the mouse was used to estimate the integrity of the capillary permeability, as described previously[15]. Briefly, mice were inoculated intravenously (i.v.) with 100 µl 2% solution of Evans blue dye dissolved in 0.85% sodium chloride, followed 5 min later with hCF i.p. After 30 min, mice were anaesthetized with ether and the peritoneal cavity was washed with 5 ml normal saline. The lavage fluid was collected, filtered through glass wool column and made up to a final volume of 10 ml. The optical density at 590 nm was determined with a spectrophotometer. The protein content of the fluid was calculated by comparison with a standard curve obtained with various concentrations of bovine serum albumin estimated by the technique of Lowry *et al.*[21]. The results were expressed as µg protein/mouse. The control mice received Evans blue dye followed by i.p. inoculation of NS and for background values mice received the dye followed by i.p. inoculation of normal saline.

Assay of the breakdown of blood-brain barrier

Mice were inoculated i.v. with 200 µl of Evans blue dye followed 5 min later with i.v. inoculation of various doses of hCF. The control mice were inoculated with NS in place of hCF. For background values equal number of mice were given dye only[16]. After 1 h mice were anaesthetized with halothene i.p. and the brain was perfused with 5 ml normal saline through the left cardiac ventricle. A 10% homogenate of the brain was prepared in normal saline and centrifuged at 4°C at 6000 *g* for 15 min. The clear supernatant was collected and its absorbance was determined at 590 nm for detection of Evans blue. Protein contents of the clear supernatant were estimated as described above.

The mean ± SD of the data obtained from 10–12 mice in repeated experiments have been presented. The data have been analysed using Student's *t* test and a *p* value of less than 0.05 was considered significant.

Results

Dot blot analysis

The representative dot blot reaction of the sera is presented in Figure 1 *a*. Analysis of the results showed that 98 out of 220 sera from various grades of dengue illness gave a positive dot blot reaction (44%). The positivity was higher in the acute phase sera (first sample) compared to the convalescent phase sera collected at the time of the discharge from the hospital (second sample). Further, the sera from cases of DHF grade 1 had highest positivity. Only 4 cases of DHF grade 4 could be studied, out of which one was positive; the number is too small (Figure 1 *b*). The diagnosis of virus isotype (dengue virus type 1–4) was available in 85% of the patients out of which 44 were dot blot

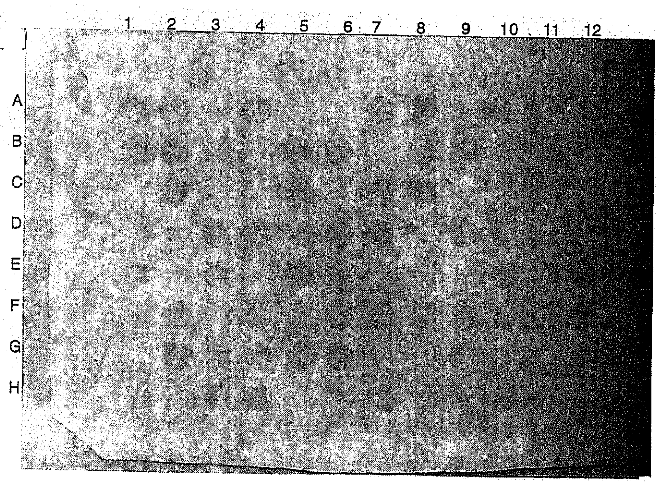

Figure 1 *a*. Dot blot test for detection of the hCF in the sera of the patients of various grades of dengue illness. The controls included cases of non-dengue illness and normal healthy individuals (lane 12, A-D, G, H). Purified mouse CF was used as positive control (lane 12, E, F).

positive (52%) and were distributed in all the four isotypes of dengue virus. The number of cases with a diagnosis of dengue virus type 2 infection was 4 only which is too small a sample size. Among the 27 control non-dengue illness sera, 2 gave positive reaction; but the diagnosis of dengue in them could not be firmly excluded.

Purification of hCF

Preliminary purification of hCF from 34 dot blot positive sera of dengue illness was done on DEAE-cellulose columns. The typical elution profile of cytotoxic activity is shown in Figure 2 *a*. One serum sample from a case of non-dengue illness was subjected to DEAE-cellulose chromatography but the cytotoxic activity was not found in any of the eluted fractions (data not presented). The eluted cytotoxic fractions from different runs were pooled peak-wise, dialysed, concentrated and then studied for dot blot reaction. Figure 2 *b* shows that all the three peaks reacted with anti-mCF antibodies giving a positive reaction, thus showing that all the three peaks had mCF-like substance. The protein from each peak, further purified on HPLC, showed a single peak of protein, having the cytotoxic activity. The final yield of the purified hCF was approximately 5 to 10 μg/ml sera in different runs.

PAGE analysis

The proteins present in the three cytotoxic peaks were analysed on PAGE. The major protein band, present in all the three cytotoxic peaks and in crude hCF obtained from the cultures of peripheral blood mononuclear cells stimulated with DV *in vitro*[7], was located in line with the single band of pure mCF (Figure 3, lane 6) and had the molecular weight of 43 kD. A similar profile was detained on SDS-PAGE silver stained gels (data not presented). This indicated that hCF and mCF have a similar molecular mass on native PAGE. The isoelectric point studied on Pharmacia Phast gel electrophoresis system showed a diffuse band at 4.5–5 pH position (Figure not presented here).

The HPLC-purified protein was electrophoresed and blotted on PVDF membrane for amino acid sequencing. The N-terminal amino acid sequencing was done by V. S. Chauhan and Aparna Gupta, International Centre for Genetic Engineering and Biotechnology, New Delhi and by H. S. Savithri, Indian Institute of Science, Bangalore. They found that N-terminal of hCF was blocked (pers. commun.). Efforts are being made to determine the sequence following cleavage of the protein.

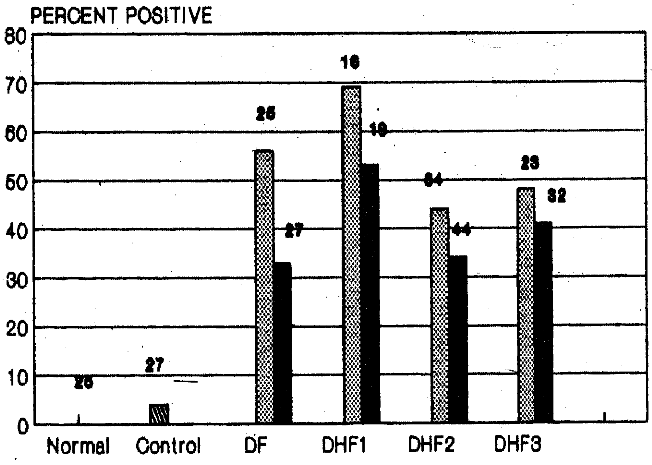

Figure 1 *b*. Summary of the results of dot blot test on the sera from cases of various grades of dengue illness, healthy individuals (normal) and the non-dengue illness (controls). From each patient, blood samples were collected on the day of admission (▨) to the hospital and at the time of discharge (■) from the hospital. The numbers above the bars denote total number of samples.

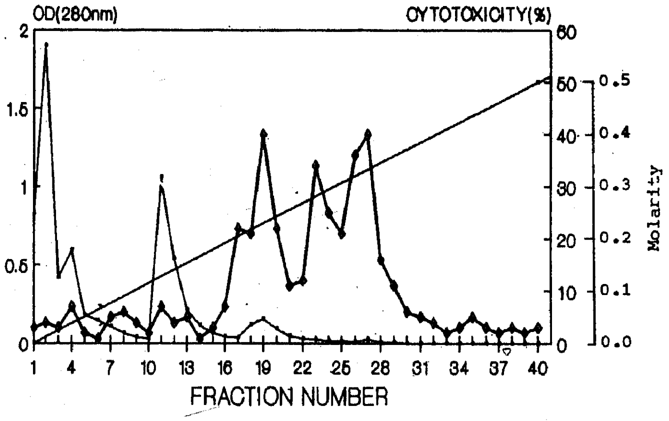

Figure 2 *a*. Elution profile of the ion exchange chromatographic purification of hCF from the sera of patients of dengue using diethylaminoethyl (DEAE)-cellulose packed columns. (•), Absorbance at 280 nm; (◊) cytotoxic activity; (—), NaCl gradient.

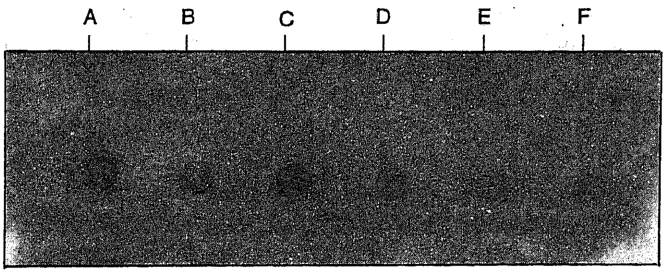

Figure 2 *b*. Dot blot reaction of cytotoxic peaks eluted in Figure 2 *a*. The findings show a positive reaction with mouse CF used as positive control (A); cytotoxic peak 1 (B); cytotoxic peak 2 (C); and cytotoxic peak 3 (D). A negative reaction was obtained with the two negative NF controls (E, F).

Western blot analysis

The Western blot analysis confirmed the findings of PAGE. The protein bands located at 43 kD position of the cytotoxic chromatographic peaks and mCF reacted equally with anti-mCF-antisera and anti-hCF-antisera (Figure 4) but not with the heterologous or normal sera (data not presented). The non-cytotoxic chromatographic fractions, NF and an irrelevant protein, the helper factor[22] used as negative controls.

Effect of hCF on vascular permeability

Groups of mice were inoculated with hCF i.p. in various doses and their capillary permeability was assayed. It was observed that administration of the hCF increased the dye-bound protein diffusion in the peritoneal cavity in a dose-dependent manner (Figure 5). With a dose of 3 μg hCF value of the dye-bound protein was 70 ± 6 μg/mouse ($p < 0.001$) while that with 25 μg it was 167 ± 8 μg/mouse ($p < 0.001$) compared to the control values. Similarly, the leakage of protein in the brain tissue was also dose-dependent (Figure 5).

In the second set of experiments the effect of duration of exposure to 5 μg of hCF on the capillary permeability was assayed. It was observed that with the peak increase in dye-bound protein leakage in the peritoneal cavity occurred after 30 min of hCF inoculation. Thereafter, the amount of protein leakage quickly declined and the vascular integrity was restored by the 4th h (Figure 6). Therefore, in further experiments the observations were recorded at 30 min after inoculation of hCF. The leakage of the protein in the brain was maximum at 1 h with 5 μg hCF given i.v. The integrity of the blood-brain barrier was restored at the 4th h (Figure 6).

Effect of anti-hCF- or anti-mCF-antisera

The specificity of the effect of hCF on the capillary permeability was investigated by pretreatment of mice with 200 μl of the antisera against hcF or CF. Mice were challenged 24 h later with 5 μg hCF and the capillary permeability was assayed. For control, mice were inoculated with normal mouse serum in place of antiserum. Figure 7 shows a dose-dependent inhibition of hCF-induced increase in capillary permeability by treatment with antisera against both hCF and CF. Similar inhibition of damage to the blood-brain barrier by the specific antibody was also observed (data not presented).

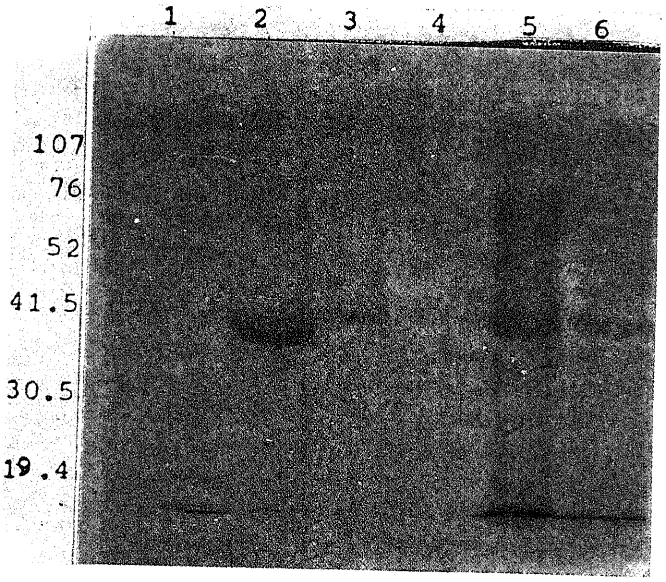

Figure 3. Polyacrylamide gel electrophoresis of hCF. The gels were stained with Coomassie Brilliant Blue R-250 stain. Lane 1, molecular weight markers; Lane 2, hCF peak 1, Lane 3, hCF peak 2; Lane 4, hCF peak 3 on ion exchange chromatography; Lane 5, crude hCF produced in human peripheral blood mononuclear cells *in vitro*[8]; Lane 6, purified mCF.

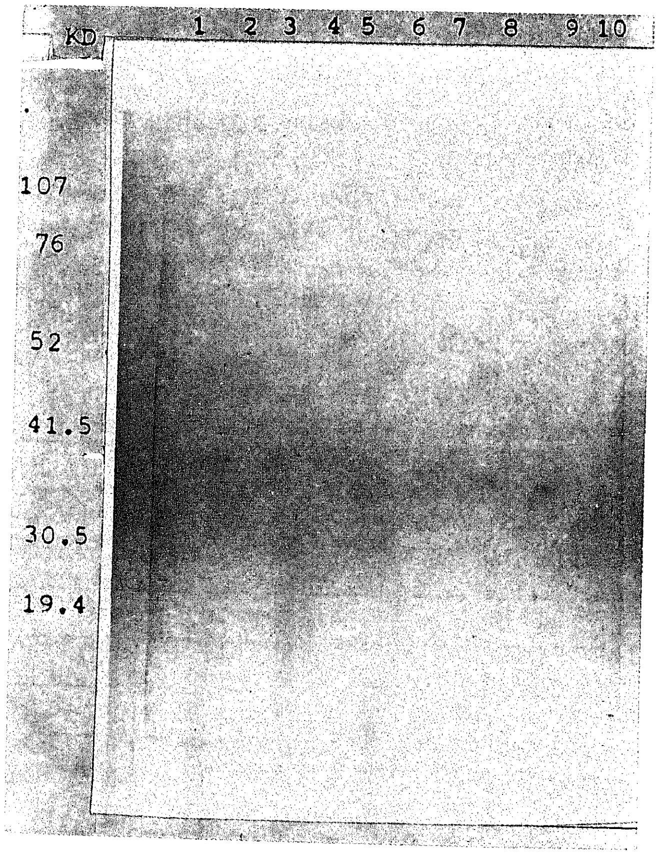

Figure 4. Western blot analysis of hCF. Lane 1, mouse NF (negative control), Lane 2, mouse CF (positive control), Lane 3, hCF peak 1 of ion exchange chromatography (IEC) further purified on HPLC; Lane 4, noncytotoxic fraction 2 of IEC; Lane 5, noncytotoxic fraction 10 of IEC; Lane 6, noncytotoxic fraction 30 of IEC; Lane 7, peak 1 of IEC; Lane 8, peak 2 of IEC; Lane 9, peak 3 of IEC, and Lane 10, noncytotoxic irrelevant protein.

Effect of anti-histaminics

Avil is known to block H1 while ranitidine blocks H2 receptors of histamine. Therefore, experiments were conducted to investigate whether the increase in vascular permeability by hCF is mediated by histamine. A group of mice pretreated with avil or ranitidine i.p., followed 30 min later with 5 µg of hCF i.p. and capillary permeability was assayed. Pretreatment of the mice with avil or ranitidine inhibited the hCF-induced increase in vascular permeability (Figure 8). Similar inhibition of hCF-induced damage to the blood-brain barrier was observed both with avil and ranitidine (data not presented).

Discussion

The findings of the present study have established the presence of dengue virus-induced cytotoxic factor (hCF) in cases of dengue illness. This was done by screening the cases of DHF by inhibition ELISA (not described here) followed by a dot blot test using anti-mCF-antisera.

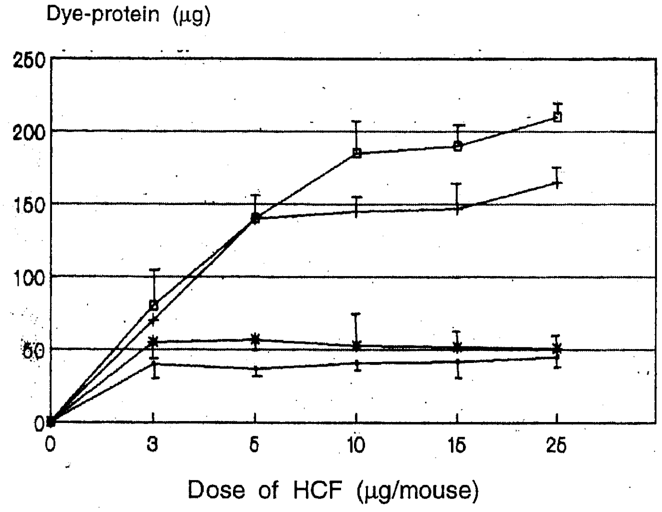

Figure 5. Dose-dependent increase of capillary permeability and breakdown of blood-brain barrier in mice inoculated with hCF. The control mice received the dye and the normal human serum proteins (NS) in place of hCF. For background values mice were given only the dye. Mice tested for capillary permeability (□) and the controls (•) and for the blood-brain barrier (+) and the controls (*).

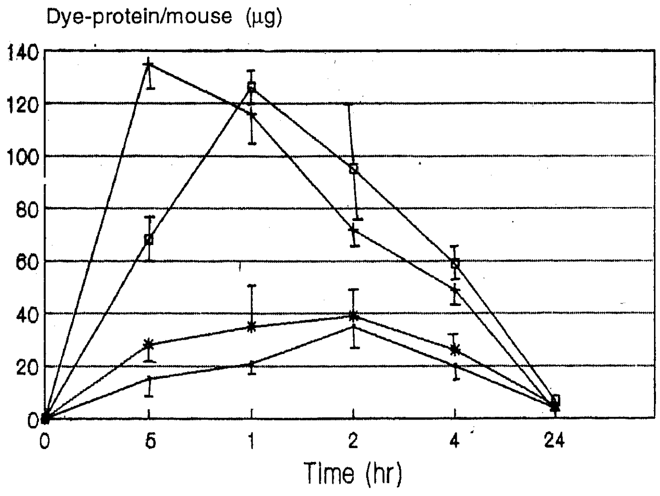

Figure 6. Effect of duration of exposure to hCF on the capillary permeability in test (+) and control (•) mice; and on the blood-brain barrier in test (□) and control (*) mice.

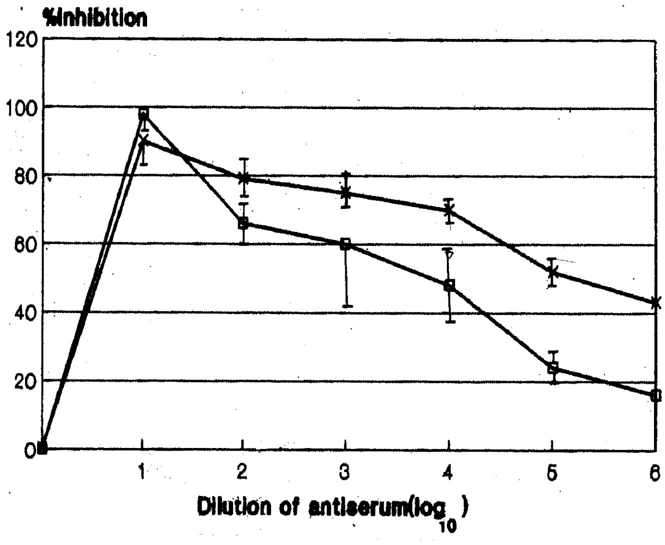

Figure 7. Effect of pretreatment of mice with anti-mCF (□) or anti-hCF antisera (×) on hCF-induced increase in capillary permeability. Control mice were given normal mouse sera in place of the antisera.

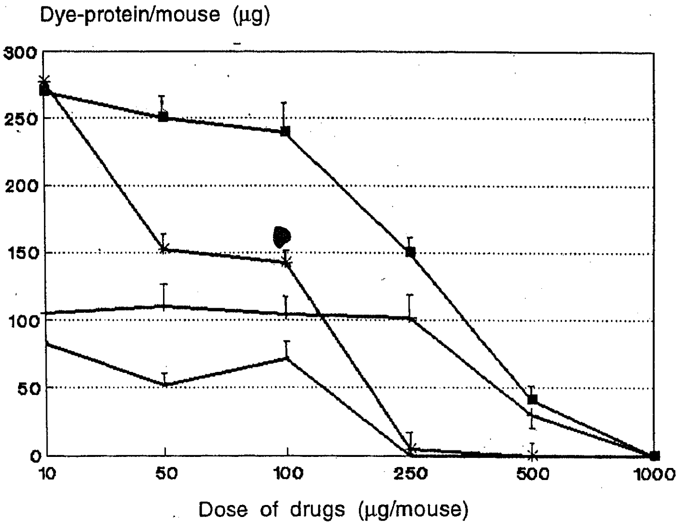

Figure 8. Effect of pretreatment of mice with anti-histaminic drugs, avil (+), or ranitidine (×) on the hCF-induced increase in capillary permeability. Control mice were not given anti-histaminic drugs. For background values mice were inoculated with avil (■), or ranitidine (*).

Finally, from the sera of 34 patients the hCF protein was purified by ion-exchange chromatography (IEC) and then by HPLC. The elution profile of hCF on IEC is similar to that of mCF reported earlier[20] – and the protein in the three peaks was similar in migration on gel, antigenicity and the biological activity as seen with mCF[20]. The purified hCF had a molecular mass of 43 kD and an isoelectric point of pH 4.5–5. The antisera against hCF reacted with mCF and hCF in a Western blot test. These findings have indicated the presence of hCF in cases of DHF.

This has been confirmed in an experimental study where we have shown that DV can stimulate human peripheral blood mononuclear cells to produce hCF; the cell responsible for its production is CD4 positive T cells. This was supported by demonstration of the presence of mRNA for hCF in T cells from the cultures by Northern hybridization[7], using oligonucleotide probe prepared on the basis of aminoterminal sequence of mCF. Another question raised was whether hCF has any pathological effect. In further experiments the pathogenicity of hCF was studied. It has been shown that mCF increases vascular permeability[16] and damages the blood-brain barrier in mice[15]. Using the same protocol it was observed that hCF increased the capillary permeability and damaged the blood-brain barrier in mice and restoration of the vascular integrity occurred in 4 h time. The increase in the capillary permeability was abrogated by pretreatment of mice with the anti-mCF- or anti-hCF-antisera and also by the anti-histaminic drugs, avil or ranitidine. This showed that hCF can be pathogenic in man.

hCF is similar to mCF in a number of characteristics like molecular mass, antigenic cross reactivity and demonstration of mRNA for both in Northern hybridization using the same oligonucleotide probe[7] and pathogenicity in mice. mCF is a pathogenesis-related protein and capable of reproducing all the lesions in mice that are seen in human cases of DHF. Therefore, the present study has raised several questions; whether hCF alone plays a role in the pathogenesis of human dengue disease, specially in the severity of the DHF, or requires the help of other cytokines such as TNF-a, IL-1, IFN-γ, IL-2 (ref. 23) or other mediators as necessary factors; if hCF is a unique protein induced by dengue viruses, can it be used for quick diagnosis of the cases, specially by demonstration of mRNA for hCF by Northern hybridization using peripheral blood mononuclear cells of the patients of DHF; and a more remote possibility, if it can be used as an 'anti-disease vaccine'[18,19] as in mouse model.

A correlation of the presence of hCF with the different grades of DHF and the different isotypes of the dengue virus is needed. Due to the small sample size of the present study it is not possible at the moment. We have studied cases of the recent epidemic of DHF and have isolated dengue type-2 virus from two cases at Delhi (cases of M. K. Bhan, AIIMS) and two cases at Lucknow and have shown presence of specific IgM in a number of cases, thus confirming the diagnosis of dengue. Study of these cases showed that hCF is present in the sera from the day 1 to 20 and not after that period (U. C. Chaturvedi *et al.*, unpublished data). Presence of hCF may depend upon the day of infection as shown in DV-infected mice[4]. This may increase the positivity of the samples; but in absence of this information it is not possible at present. The tests for hCF need refinement by using specific monoclonal antibodies and more epidemiological and experimental studies are needed. As the N-terminal of hCF is blocked and is not available for amino-acid sequencing, it could not be matched with mCF or other similar cytokines to look for any homology. hCF is similar to mCF in most of the properties including molecular mass and detection of their mRNA by Northern hybridization using the same probe. The cDNA library for mCF has been prepared and a vigorous attempt is being made to clone the gene for hCF. This will help in proper placement of hCF in appropriate group of cytokines.

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Thermotolerance and the heat shock response in *Musca domestica*

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The relative abilities of larvae, pupae and adults of a laboratory population of the tropical housefly, *Musca domestica*, to withstand increasing environmental temperature (37°C to 48°C) were studied in terms of per cent adult survival/emergence. Pupal stage appeared best adapted to higher temperatures (44°C or 46°C), exhibiting significantly higher per cent survival as adult. In contrast, the tolerance of adult flies to higher temperature shock (44°C or 46°C) was poorer. A short period (1 h) of pre-conditioning at 37°C resulted in the development of considerable thermotolerance to higher temperatures (42°C to 46°C). Eight prominent heat shock polypeptides of approximate molecular weights 80, 73, 70, 64, 30, 28, 26 and 20.5 kD were induced upon *in vitro* exposure of the larval or adult tissues at 42°C to 46°C temperature. The 70 kD polypeptide appeared predominant in all the tissues at all temperatures. The pattern of expression (number and relative intensity) of various heat shock proteins at different temperatures of heat shock appeared to be related with the observed per cent values of adult survival at corresponding temperatures. Possible role of the heat-inducible proteins in thermotolerance and adaptation to severe seasonal fluctuations in environmental temperature has been discussed.

STRESS response is considered to be the most conserved biological mechanism for maintaining cellular homeostasis during periods of normal cell growth and differentiation and during stresses caused by environmental temperature, heavy metals, amino acid analogues, certain pathophysiological states (e.g. inflammation, infection, ischemia, fever), etc[1–3]. The heat shock proteins (HSPs), which are specifically synthesized upon exposure of the cells to such environmental insults, are considered to play a key role in keeping the cells composed during transient stresses[3–6]. A brief exposure to mild heat stress prior to severe heat shock leads to development of thermotolerance[1,7], and hence an increased survival at lethal temperatures[8–11].

In recent years, considerable interest has emerged on the evolutionary significance of environmental temperature which has an important bearing on ecological adaptations[12]. Temperature is considered as an important environmental factor that influences habitat selection and thus the species distribution, especially in ectotherms[12,13]. The heat shock response has been assigned significant roles in development of thermotolerance and thermal adaptation in tropical conditions where seasonal fluctuations in ambient temperature are in extreme range as in mid-summer[11,14,15]. Our understanding of the regulatory mechanism of thermal adaptation to ambient temperature is still in infancy. The problem of thermoregulation can be more serious in ectotherms whose body temperature is equilibrated with the environmental temperature and might lead to severe consequences if not immediately responded to[12]. The mechanisms of cellular adjustments to sudden and transient thermal shocks involved in natural conditions and that active in *in vitro* cell cultures may not necessarily follow similar kinetics. Detailed probes to find different characteristics of heat shock response at the organismal level and in natural or as mimicked thermal environment will augment the basics of the underlying mechanisms. Insects, particularly flies, have been regarded as one of the most suitable animal models for such investigations owing to their shorter life span and greater potential to adapt to a wide range of environmental stresses.

This report is based on our observations in respect of (i) the assessment of the relative levels of tolerance of tropical *M. domestica* to wide range of environmental temperatures, as experienced during summer months at different stages of development and (ii) the cellular mechanism to counteract thermal injury caused by extreme ambient temperature.

Materials and methods

Fly culture

An established colony of *M. domestica* reared in the insectory at 24°C–26°C was used for the experiments.

Thermotolerance

Young flies (2 days post eclosion, $n = 15$), pupae (pharate adults, $n = 25$) and larvae (third instar wandering stage, $n = 25$) were separately placed in thin-walled glass tubes and given heat shock in water baths set at 37°C, 42°C, 44°C, 46°C and 48°C for 30 min to 180 min, directly or after pre-treatment for 1 h at 37°C. Each set was taken in triplicate. After heat shock they were allowed to recover at normal temperature and the surviving flies were counted to assess survival percentage ($\% \pm$ SE). Parallel sets of unstressed individuals were taken as controls.

Physiological response

Tissues from third instar wandering stage larvae (salivary glands and Malpighian tubules) and adults (brain, Malpighian tubules, ovaries and testes) were excised in Poels' salt solution[16] and heat shocked for 1 h at various temperatures as mentioned above. During the last 30 min of the heat shock, incubation medium was replaced with pre-warmed fresh medium containing ^{35}S-methionine (Act 100 μCi/ml, BRIT, Bombay). Tissues were dissolved in sample buffer (50 mM Tris.HCl, pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT, 2mM PMSF and 0.1% bromophenol blue) by boiling in a water bath for 5 min. For comparison, labelled heat shocked (1 h, 37°C) samples of larval salivary glands or adult ovaries of *Drosophila melanogaster* and control unstressed larval and adult tissue samples from *M. domestica* were also prepared as above.

Labelled polypeptides were separated on discontinuous SDS-PAGE and the vacuum-dried gels were processed for fluorography as described earlier[11]. The approximate molecular weights of the heat shock proteins (HSPs) were calibrated with reference to the co-migrating standard molecular weight markers (Sigma, USA) and *Drosophila* HSPs in the parallel lanes.

Results and discussion

Thermotolerance during development

The level of tolerance of *M. domestica* larvae, pupae or adults to varying environmental temperature was recorded as per cent adult emergence (for larva or pupa) or adult survival (for adults, regaining of normal activity following recovery). Exposure of the larvae, pupae or adults to 37°C for even a prolonged period (more than 4 h) had no visible effect, however, 42°C (1 h or more) was found critical to the adults but not for the larvae or pupae (Figure 1 *a–c*). Increase in heat shock temperature to 44°C or above (46°C–48°C) severely affected survival at all stages of development (Figure 1). Even a very short exposure at 48°C (15 min) resulted in a total mortality (data not shown). Pre-treatment of the larvae, pupae or adults to 37°C prior to heat shock at 42°C, 44°C or 46°C, considerably increased the number of surviving individuals as active adults (Figure 1). In case of larvae or adults, however, it was observed that preconditioning did not cause any significant change in survival once the extent of thermal stress was significantly above the tolerance level (e.g. 46°C for larva and 44°C for adult; Figures 1 *a* and *c*). In contrast, pupae were found much more tolerant to higher temperature shock (44°C or 46°C) and exhibited significant thermotolerance when preconditioned (Figure 1 *b*). The hard external puparia acting as an effective protective shield and significantly low metabolic status are likely to account for such a high level of thermotolerance in pupae. Earlier observations in *Chironomous*[14] and *Lucilia cuprina*[11] also reported pupae to be much better adapted to higher ambient temperature compared to the larvae or adults. Thus the present findings on the relative levels of adult survival or tolerance during development at varying degrees of thermal stress demonstrate the potential of *M. domestica* to withstand a wide range of thermal fluctuations as experienced in the field during the summer months.

Heat shock proteins

Two of the major tissues selected for analysis of the heat shock proteins are the salivary glands (larva) and Malpighian tubules (larva and adult). Both are known to be functionally most active tissues. Adult brain was taken as a differentiated non-mitotic cell type. Testis and ovary are the reproductive organs and disruption in their function might affect fecundity and, to a certain degree, the survival. Mild heat shock partially inhibits general protein synthesis but high temperature shock affects appreciably the ongoing protein synthesis. The selective and increased synthesis of the heat shock proteins during severe temperature shocks therefore, appear to have a significant role in stabilizing the general translational machinery during the period of heat stress[18].

A total of eight prominent heat inducible polypeptides were observed in various larval and adult tissues examined. The approximate molecular weights of the common polypeptides were 80, 73, 70, 64, 30, 28, 26 and 20.5 kD. Though the presence of a low level of 73, 28, 26 and 20.5 kD HSPs was also noted in unstressed control tissues, their intensities were found to be increased considerably upon heat shock (Figures 2 and 3). Notably,

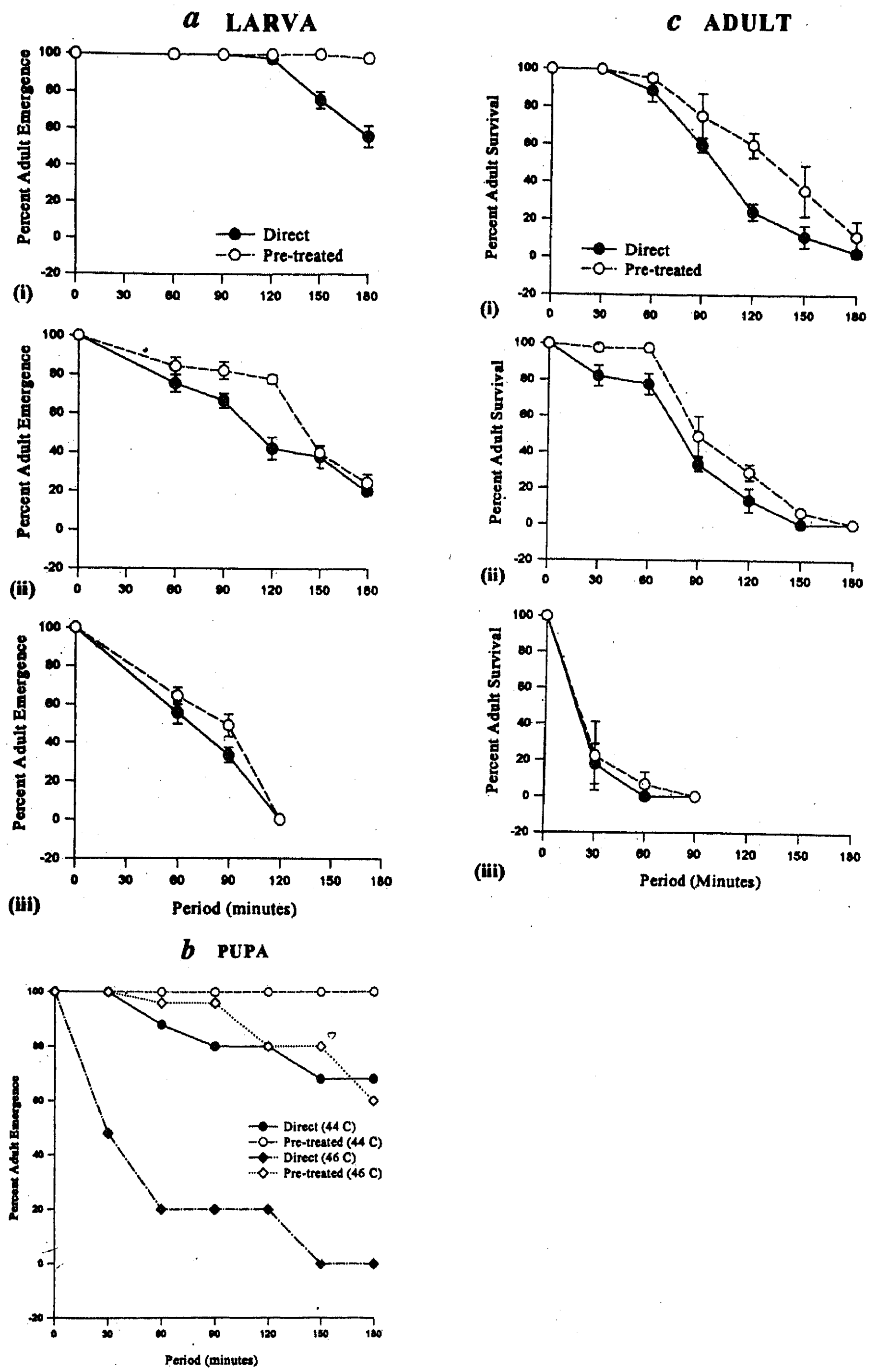

Figure 1. Per cent adult emergence (for larva and pupa) or survival (for adult) of *Musca domestica* following heat shock to larva (*a*), pupa (*b*) or adult (*c*) at 42°C (*a*-i and *c*-i), 44°C (*a*-ii, *b* and *c*-ii) or 46°C (*a*-iii, *b* and *c*-iii) for various periods of time (30, 60, 90, 120, 150 and 180 min), directly or after pre-treatment at 37°C (1 h).

the major low molecular weight HSPs, such as 20.5, 26, 28 and 30 kD, in most of the larval (Figure 2 *a*) and adult (Figure 3) tissues, appeared as prominent as 70 kD HSP at most of the heat shock temperatures. The optimal level of induction of most of these HSPs was noted at 42°C/44°C; it was much less at 46°C or 48°C except for some of the major HSPs like 80, 73 and 70 kD (Figures 2 and 3). Among these, the 70 kD polypeptide appeared as the most abundant class of HSPs and may possibly correspond to the HSP70 of *Drosophila* and other organisms. Immuno-characterization of this protein will more conclusively establish homology.

In addition to the common HSPs, larval salivary glands exhibited distinct expression of a 40 kD polypeptide (37°C–44°C) and two low molecular weight HSPs of 19 kD (at 37°C) and 18 kD (at 42°C) whose expression appeared not only specific to salivary glands but also was temperature dependent (Figure 2 *a*). Low-level expression of several other polypeptides of molecular weights 62 kD (37°C–44°C), 59 kD (37°C and 42°C), 30 kD (37°C) and 29 kD (37°C and 42°C) were also noted (Figure 2 *a*). None of these was observed at 46°C or 48°C. In addition, a 24 kD heat-inducible polypeptide was noted only at 37°C and 42°C. Its level, however, appeared relatively higher at 42°C than at 37°C.

Larval Malpighian tubules, however, showed a somewhat different pattern of expression of HSPs. The three major HSPs, 80, 73 and 70 kD were noted maximally induced at 42°C/44°C (Figure 2 *b*). Significant expression of a 52 kD polypeptide was also noted. Interestingly, unlike in other tissues, instead of 73 kD HSP, synthesis of 70 kD HSP, was seen to be completely repressed at 48°C. Whether the 73 kD HSP is an isoform of 70 kD

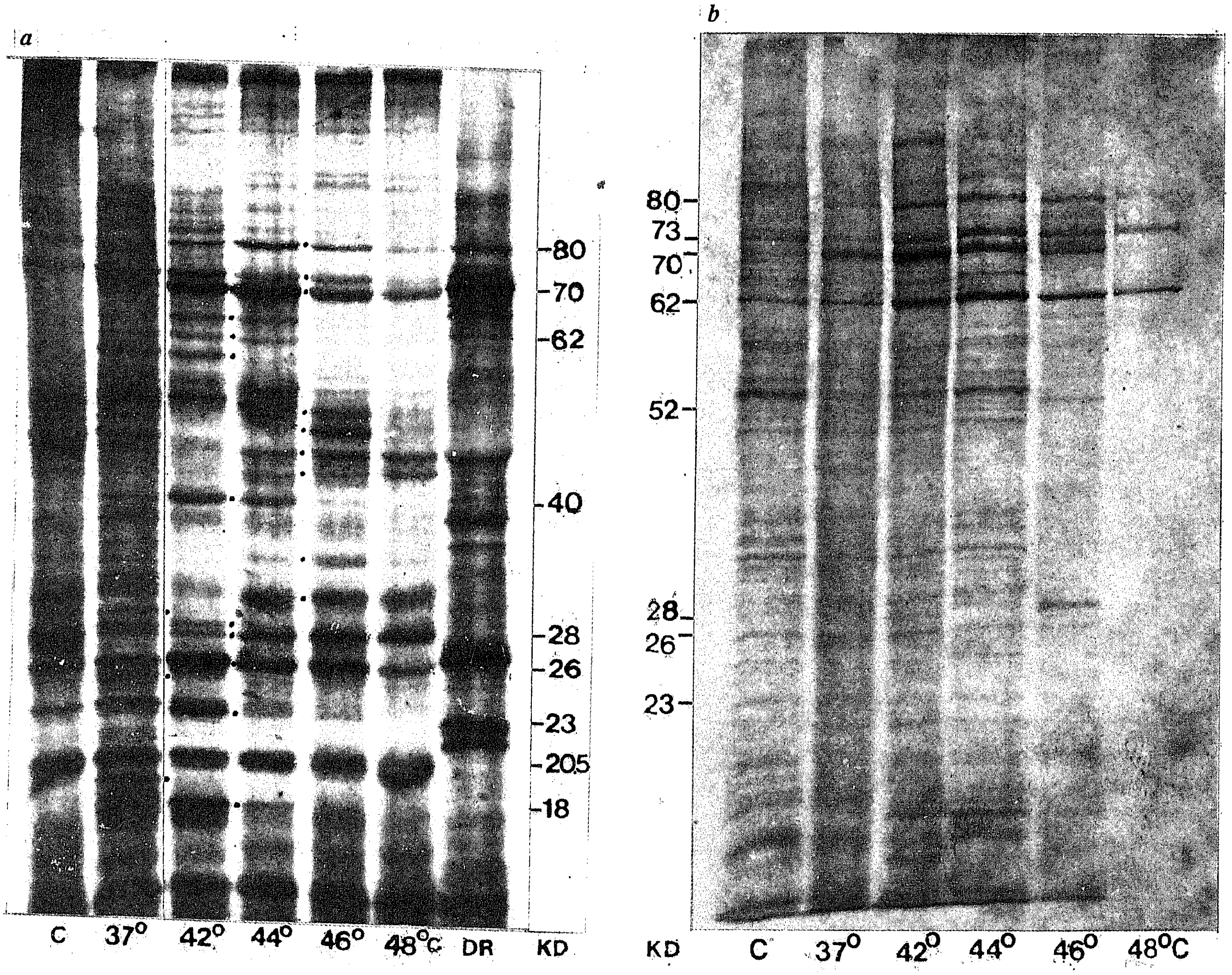

Figure 2. Synthesis of heat shock proteins in *M. domestica* larval salivary gland (*a*) and Malpighian tubule (*b*), heat shocked (1 h) at various temperatures (37, 42, 44, 46 and 48°C). For molecular weight reference, heat shocked (37°C/1 h) sample of *Drosophila* larval salivary gland is shown in lane DR (*Drosophila*; *a*). Approximate molecular weights are indicated in kD.

HSP and is relatively more thermostable than HSP70 in larval Malpighian tubules at higher temperatures, needs further investigation. Notably, a major (constitutive) protein of approximately 62 kD was synthesized at all temperatures at elevated levels in larval Malpighian tubules and even appeared more prominent than the 73 kD HSP at 48°C (Figure 2 *b*). Significantly, the level of low-molecular weight (LMW) HSPs in larval MTs was lower than that in the larval salivary glands or in either of the adult tissues. The general pattern of the HSP induction at 43°C in the adult Malpighian tubules was observed similar to that in the other tissues except that the synthesis of the 20.5 kD was reduced compared to other LMW HSPs. In this tissue two polypeptides of 62 kD (constitutive) and 64 kD (heat inducible) appeared to be very significantly labelled (Figure 3).

The general pattern of induction of HSPs at 43°C in the adult brain tissue was similar to that in the other tissues (Figure 3). The adult ovary or testis, heat shocked at 43°C, exhibited significant expression of all the HSPs as observed in larval salivary glands. Interestingly, the 64 kD protein showed an increased level of expression, particularly in the testis. However, the 62 kD polypeptide could not be detected either in the testis or in the ovary. No sex-specific variation in the HSPs could be found except that the 30 kD HSP appeared absent in the ovary while being a prominent LMW HSP in the testis (Figure 3). Selective and significant expression of the HSP64 in the adult tissues thus appears to be developmental stage (adult) specific.

The pattern of induction of different HSPs (number) and their relative levels at various temperatures (42°C to 48°C) was generally found to be related to the per cent adult survivals at the corresponding temperatures. Positive correlation between the cellular concentration of the HSP70 and the degree of induced thermotolerance has also been reported for the cells in culture[7,19–22]. In a recent observation on an intertidal mussel *Mytilus trossulus*[23], seasonal increase in the level of the endogenous HSPs has been indicated to confer thermotolerance. It should be noted that apart from the HSP70 and some other high molecular weight HSPs (e.g. HSPs 60 kD and 104 kD), role of certain low molecular weight HSPs (e.g. 28 kD and 30 kD) in the development of thermotolerance has also been implicated[7,24–26].

Pre-exposure to mild heat shock temperature is shown to induce a low level of some of the major heat shock proteins (as also noted in this study, see HSPs at 37°C), which are suggested to generate thermotolerance at higher temperature[1,8,17]. The natural populations of housefly that

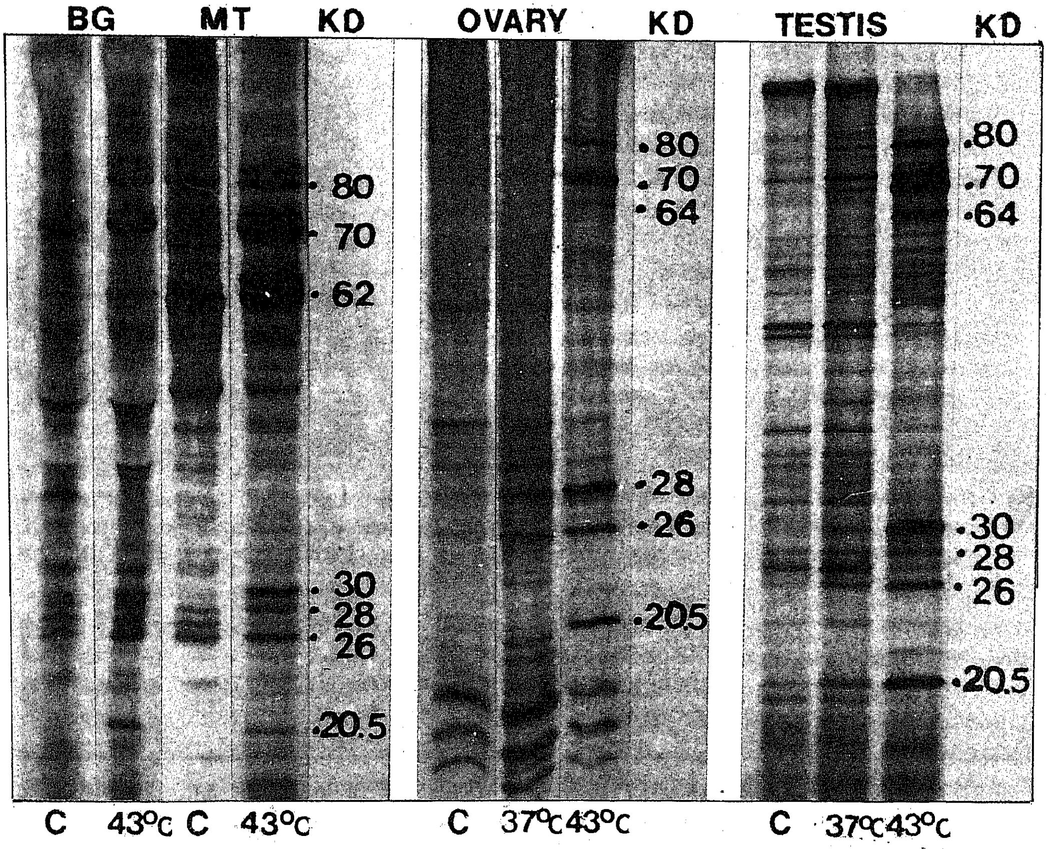

Figure 3. Synthesis of heat shock proteins in *M. domestica* adult brain, Malpighian tubule, testis and ovary heat shocked (1 h) at 37°C and 43°C. Approximate molecular weights are indicated in kD.

remain exposed to a wide range of thermal fluctuations during summer months, ranging from mild to severe heat shock conditions, may therefore, be capable of developing better resistance against severe ambient temperature.

The physiological response to heat shock in *M. domestica*, appears to be generally comparable to that observed in *Drosophila* or *L. cuprina*[11], except for some differences which may be attributed to species-specific differences in their genetic, behavioural and morphological characteristics.

Though the general pattern of HSPs in *L. cuprina* and *M. domestica* appeared similar, certain interesting differences were quite evident. Unlike in *L. cuprina*, where the Malpighian tubule-specific 62 kD (a homologue of HSP60 family) and 64 kD species of polypeptides were constitutive and not much affected by temperature shock[11] in *M. domestica* these polypeptides were heat inducible. Further, while the 62 kD polypeptide was Malpighian tubule-specific, the 64 kD HSP was seen, in addition to the Malpighian tubules, in adult reproductive tissues as well, although this protein was absent in the adult brain. In *Drosophila* also, a 64 kD polypeptide was reported to be induced specifically by heat shock in Malpighian tubules only[27,28]. This polypeptide was found homologous to the HSP60 family proteins and although induced by heat shock only in the Malpighian tubules, it was constitutively present in all the tissue types of *Drosophila*[29]. Though immunological identities of the 62 and 64 kD HSPs in *M. domestica* are currently not known, we expect at least one of them to be a member of the HSP60 family. Significance of their tissue specificity remains to be examined.

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Optical limiting action in poly(para-methoxy phenylacetylene)

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Optical limiting action is demonstrated at 532 nm with unfocused nanosecond laser pulses in poly(para-methoxy phenylacetylene) in the solution state with a threshold limiting fluence of 65 mJ/cm^2.

POLY(para-methoxy phenylacetylene) is a conjugated polymer with carbon atoms on the backbone, and hydrogen and para-methoxy groups as the sidegroups. Conjugated polymers are known[1] to have high values for the susceptibility tensor $\chi^{(3)}$ due to the presence of the delocalized π-electrons. In this communication, the method of preparation of this polymer sample is given followed by a discussion of the linear and nonlinear optical studies and the results.

The method of preparation is as follows: A 50 ml single-necked round-bottomed flask with magnetic stirring bar, capped with a rubber septum, is flame-dried and under nitrogen atmosphere, $Mo(Co)_6$ (0.145 g, 0.54 mole) is taken in the flask and about 6 ml of toluene is added. The resulting solution is refluxed for 6 h in an oil bath and cooled. Para-methoxy phenylacetylene (1.45 g, 10.9 mole) is added and refluxed with stirring for 24 h. The reaction mixture is cooled and the polymer is precipitated by pouring into excess ethanol. It is filtered, washed with hot methanol and dried under vacuum. The yield is 0.68 g (47%) of dark brown polymer in amorphous powder form.

The polymer dissolves well in organic solvents. The as-prepared solution is yellowish brown when the solvent is 1,2-dibromoethane and yellow when dissolved in toluene. Accordingly, the absorption edge has a longer tail in the visible region for the former sample. The polymer is stable at room temperature. The IR spectrum of the sample taken in a KBr pellet shows the characteristic adsorptions at 3200 cm^{-1} (C–H stretch), 1600 cm^{-1} (benzene double bonds), 1504 cm^{-1} (backbone conjugation), 1456 cm^{-1} (trans-rich nature), etc.

When illuminated with the intense Q-switched second harmonic radiation at a wavelength of 532 nm from a frequency-doubled Nd : Yag laser (Quanta-Ray, DCR 2(10)), the solution prepared in 1,2-dibromoethane turns to dark brown under visual observation. The optical absorption of this laser-treated sample shows the formation of a new band in the visible region centred around 550 nm. When the solution is prepared in toluene, there is change neither under visual observation nor in the recorded absorption spectrum after treating with 532 nm laser pulses.

The formation of the band at 550 nm under 532 nm excitation is observed if the polymer is dissolved in other halogenated solvents like CCl_4 and $CHCl_3$ and polar solvents such as dimethylsulphoxide and tetrahydrofuran. However, the optical absorption of the laser-treated polymer powder dissolved in 1,2-dibromoethane failed to show the new band. This is indicative of solvent effect in the formation of the band at 550 nm. That the change could be due to a charge transfer effect is suggested by the observation of a small band at 550 nm even without the excitation at 532 nm when the polymer is dissolved in the acceptor solvent nitrobenzene. The band however remains when this solution is excited at 532 nm. If the polymer is extracted from the laser-treated solution in 1,2-dibromoethane, where the new band had been observed, and then redissolved in toluene, there is no band at 550 nm before and after 532 nm excitation. This is also indicative of the solvent effect and leads us to conclude that there is no permanent change in the polymer as far as the new band at 550 nm is concerned.

The ^{13}C and ^{1}H NMR spectra are taken for both the as-prepared polymer and the polymer extracted from the solution excited at 532 nm in the solvent 1,2-dibromoethane. The as-prepared polymer shows bands corresponding to fused cyclobutane rings in addition to those of backbone carbons and the para-methoxy groups. The band due to the fused cyclobutane rings is not present for the laser-excited sample. The thermal gravimetric analysis and molecular weight measurements of the two samples, however, show no significant differences.

Q-switched laser pulses at 532 nm are incident on the polymer solutions contained in 1-mm thick quartz cuvettes. The value of the optical absorption coefficient measured with very low intensity laser pulses is different from the values obtained from the spectrophotometer which has an incoherent source. The incident and transmitted laser pulse energies are measured and a plot of transmittance (the ratio of the transmitted to incident energies) versus the incident energy indicates a reduction in transmittance with increase in intensity above a threshold value. This is indicative of optical limiting action which is an effect opposite to that of saturable absorption.

The basic requirements of an optical limiter[2] are an excited state absorption cross section that is higher than the ground state absorption cross section, a fast intersystem crossing rate with the presence of a metastable state and the duration of the exciting pulse lower than the excited state lifetime, so that there is a cumulative

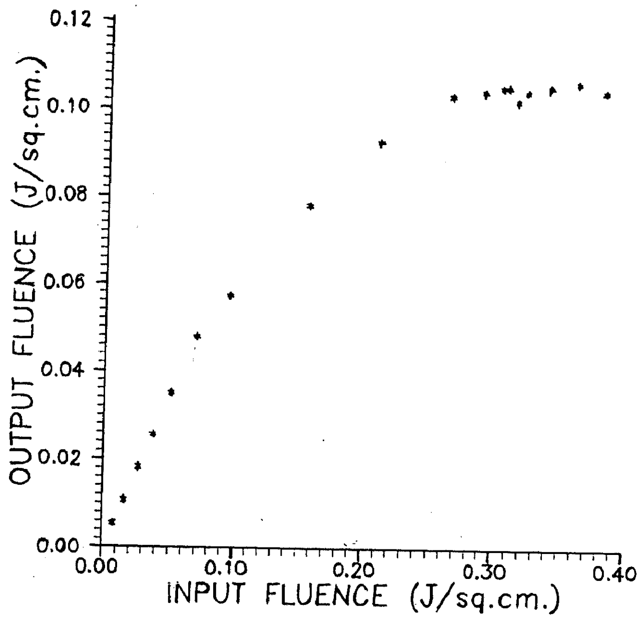

Figure 1. The variation of output fluence with input fluence in solution of poly(para-methoxy phenylacetylene) in the solvent 1,2-dibromoethane for excitation under a Q-switched doubled Nd : Yag laser.

buildup of population in the excited state, leading to increased levels of absorbance for the pumping beam.

Figure 1 shows variation of output fluence with different values of input fluence, in J/cm^2. The relation starts differing from linearity when the input fluence is 65 mJ/cm^2, ultimately clamping the output fluence at a fixed value when the input fluence reaches 250 mJ/cm^2. In comparison, C_{60} is known[3] to show a change from linearity in transmittance when the input fluence reaches 100 mJ/cm^2 for a sample with an initial transmittance of 63%. Our sample had an initial transmittance value of 69%. The variation in transmittance during an increase and a decrease in the incident intensity enables us to see the extent of recovery of the initial transmittance values. The results are indicative of minimum photodegradation of the samples. It is found that the recovery of the initial transmittance is not degraded due to repeated illumination under the laser pulses. There is no change in transmittance when the sample is illuminated with microsecond pulses at 532 nm from the same laser under non-Q-switched conditions which is due to the lower peak power of these pulses.

The third-order susceptibility $\chi^{(3)}$ as found from the optical phase conjugation experiment in a degenerate four-wave mixing geometry[4] is $1.2 \times 10^{-18}\,\mathrm{m^2V^{-2}}$. It is found that the value of the phase conjugate reflectivity continues to increase with an increase in pump intensity even in the region of optical limiting. This is another indication that the limiting action is not a consequence of sample degradation but is a true optical effect. No change was observed in the spatial and temporal profiles of the transmitted laser pulse in comparison to the input pulse, suggesting that the effect of nonlinear scattering may be minimal unlike in C_{60} (ref. 5).

In conclusion, it is found that poly(para-methoxy phenylacetylene) acts as an optical limiter at 532 nm under near resonant conditions. The threshold for limiting occurs at a fluence of 65 mJ/cm^2 and is easily realizable for practical applications. The changes in transmittance are more due to induced transitions than from thermal effects. Photodegradation of the sample is minimal as inferred by conducting nonlinear transmission studies both on freshly prepared samples and well-illuminated samples. High values of phase conjugate reflectivity obtained beyond the limiting threshold also emphasize this point. Though the solvent effects are present in the linear absorption for excitation with 532 nm laser pulses, the limiting action is not solely governed by the solvent as seen from the identical trends in the variation of high intensity transmittance for the polymer when dissolved in different solvents.

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Helix-sheet interconversion in a synthetic pore lining peptide derived from a designed ion channel

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We have designed a four-helix protein that is expected to tetramerize in the membrane to form an ion channel with a structurally well defined pore. A synthetic peptide corresponding to the channel lining helix facilitates ion transport across liposomal membranes and largely helical in membranes. Detailed circular dichroism studies of the peptide in methanol, water and methanol–water mixtures reveal that it is helical in methanol, β-structured in 97.5% water and a combination of these two structures at intermediate compositions of methanol and water. A fluorescence resonance energy transfer study of the peptide shows that the peptide is monomeric in methanol but undergoes extensive anti-parallel aggregation in aqueous solution.

THE three-dimensional structure of a protein is dictated by the sequence of its constituent amino acids, but it has not thus far been possible to develop reliable algorithms leading from a primary sequence to its three-dimensional structure. One approach to this problem has been to design proteins that would adopt a specified structure. We have designed a four-helix protein, SYNCHAN, that should form non-selective, ungated pores in membranes (Seth *et al.* in preparation). Faces of helices that interact extensively in the bacteriorhodopsin (bR) structure[1] have been used to design specific helix–helix interactions while the channel liner is an amphipathic helix based loosely on the alamethicin–zervamicin family of fungal peptide antibiotics[2]. SYNCHAN has been designed to tetramerize in the membrane, each subunit contributing a helical segment to line an aqueous pore. The pore thus formed would be stabilized by helix–helix interactions within and between subunits. Thus, segments corresponding to each of the helices should partition effectively into membranes and interact with each other in a stereospecific manner. In addition, the channel-lining segment should be able to self-associate in a manner resulting in transmembrane ion channels.

The protein, SYNCHAN, should serve as a model for *Shaker* type potassium channels which are also tetramers[3,4], each of whose subunits has six putative transmembrane segments[5–7]. Details of the design will be presented elsewhere (Seth *et al.*, in preparation). Briefly, helix–helix interactions have been designed based on interacting faces of helices in the bR structure[1]. Figure 1 *a* presents a cross-sectional view of the channel, where helix 3 is the channel liner. Faces of helix 3 interacting with helices 2 and 4 have been based on the bR structure while the surface exposed to the aqueous pore is based loosely on the alamethicin–zervamicin family[2]. The sequence of SYNCHAN is presented in Figure 1 *b*. Despite the reliance on bR for the design of interacting faces of helices, the overall sequence bears less than 25% homology to bR. We have synthesized a peptide, PCH3, corresponding to the channel lining segment (helix 3) by solid phase synthesis on a semi-automatic solid phase peptide synthesizer (LKB Biochem-Biolynx 4175). A part of the peptide was acetylated at its N-terminus, using acetic anhydride[8] before cleavage from the resin. The peptide was checked for purity by analytical HPLC (Shimadzu SPD6A) on a C18 reversed phase

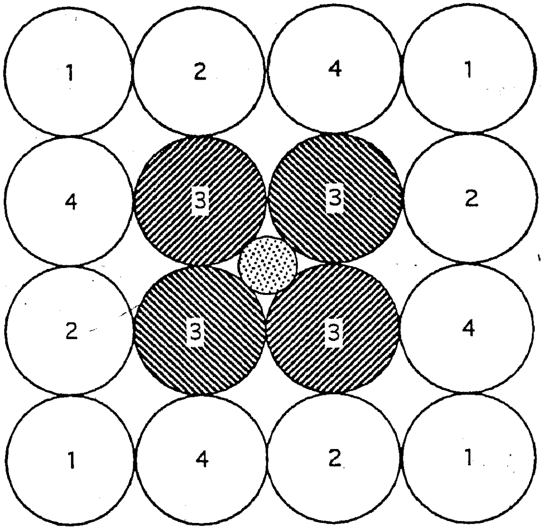

Figure 1 ***a*****.** The synthetic channel is designed to be a tetramer of a protein with 4 transmembrane helices, one of which (no. 3 here) lines the channel. The cross section of the assembled tetramer is shown, with the channel liner hatched. The central pore is also indicated in this panel.

Amino Acid Sequence of The Designed Protein

Intracellular Segment	Membrane Spanning Segment	Extracellular Segment
	#1	
NH_2-MAQHHHHHHRSR ⇨	⇨ TILYLVTAAIFIGFGLVFALTT ⇨	⇨ NVDSDQLY ⇨
	#2	
⇨	⇨ FFRVFWLLAYTALLYLLLVLYF ⇨	⇨
	#3	
⇨ KGDQGYRA ⇨	⇨ YCFALQFAPSLITFTVFLLTLW ⇨	⇨ EGDSDQLY ⇨
	#4	
⇨	⇨ AVETLLFLGAALMILGAFVDAV ⇨	⇨
⇨ KSLTDV-COOH		

Figure 1 ***b*****.** The sequence of the protein SYNCHAN.

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‡For correspondence.

Vydac column. Two-dimensional NMR studies (400 MHz) of the peptide confirmed its composition and sequence.

Transport of calcium across liposomal membrane was assayed as described in Nagaraj *et al.*[9]. Influx of calcium from the suspending medium was detected by an increase in the fluorescence of chlortetracycline (CTC) entrapped in liposomes. A rise in the intensity of CTC fluorescence on adding the ionophore requires that CTC and Ca^{++} are in separate compartments prior to the addition of the ionophore. The observed rise on adding a known calcium ionophore, X537A, thus serves to establish that the liposomes are intact (Figure 2 *a*). Introduction of AcPCH3 from a methanolic stock at 3.75 μM results in a rise in CTC fluorescence comparable to that seen with 20 μM X537A demonstrating the efficacy of the peptide in mediating ion transport (Figure 2 *b*). The kinetics of the rise in fluorescence intensity reflect the kinetics of channel formation rather than ion transport as the latter is very rapid. Interestingly, the efficiency of ion transport decreases dramatically if the peptide is delivered from a stock in methanol and water (1 : 1 v/v). In order to find out why this is so, we have investigated the structure of the peptide in methanol–water mixture of varying composition by circular dichroism (CD) spectroscopy.

The CD spectrum of the peptide dissolved in methanol exhibits minima at 224 and 209 nm characteristic of an α-helical conformation (Figure 3). On the other hand, the spectrum of the peptide diluted into HEPES buffer from a methanolic stock has a single minimum at 215 nm characteristic of β-structure (Figure 3)[10]. Addition of a methanolic stock solution to 1,2-dimyristoyl-*sn*- glycero-3-phosphatidylcholine (DMPC) liposomes results in a largely helical structure although CD band intensities are lower than those in methanol (Figure 3). It should be noted that CD spectra of small peptides containing aromatic residues reflect the algebraic sum of contributions from the peptide bonds and from the aromatic residues[11,12]. Consequently, estimates of fractional helicity from these spectra should be viewed with caution. We have not, therefore, used curve deconvolution techniques to further analyse the spectra. However, for a given peptide, alteration of CD spectra on going from one solvent to another should reflect changes in its secondary structure.

The spectrum of the peptide in mixed methanol/water systems depends on the solvent composition. Instead of using curve deconvolution programs, we have plotted the ratio $[\theta]_{222}/[\theta]_{215}$ against percentage of water in the mixed solvent system (Figure 3, inset). A decreasing

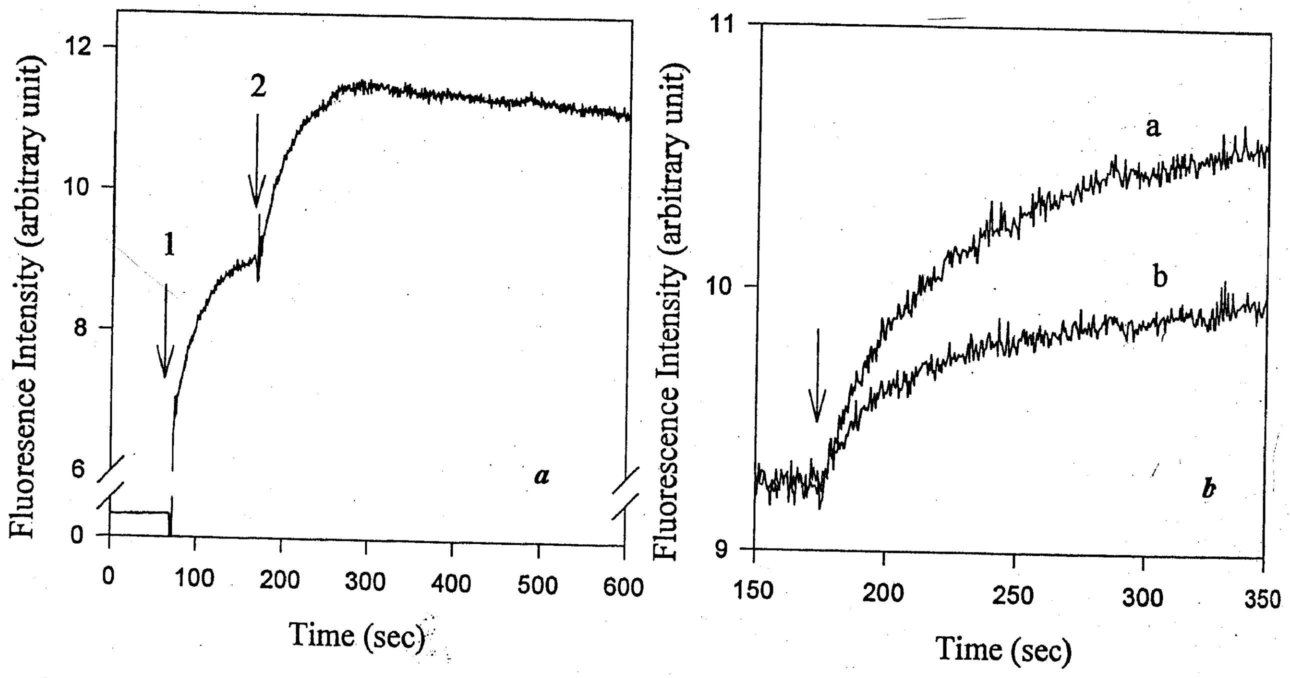

Figure 2. Ion transport across liposomal membranes. Ion transport was assayed fluorimetrically on a Spex Fluorolog 2 as described in ref. 9. 100 μl of the liposome suspension (15 mg/ml) was taken in a 3 μl fluorescence cuvette along with 10 μl of 2.3 mM CTC solution in deionized water. After incubation for 2–3 min, the mixture was diluted with 1.8 ml of buffer and stirred continuously while monitoring at $\lambda_{ex} = 400$ nm and $\lambda_{em} = 530$ nm. 50 μl of 40 mM $CaCl_2$ solution was added after collecting a baseline. Test samples were delivered through an injection port as a methanolic or aqueous solution after a steady state had been achieved. An influx of calcium into the liposomes mediated by the injected sample can be detected as an increase in the intensity of CTC fluorescence. *a*, Ionophore activity of the calcium ionophore, lasalocid (X537A). $CaCl_2$ (1 mM) is added at the point indicated by arrow #1; arrow #2 indicates the addition of the ionophore (20 μM). *b*, Ion transport activity of (a) AcPCH3 (3.75 μM) from a methanolic stock and (b) AcPCH3 (3.75 μM) from a stock in methanol/water mixture (1 : 1 v/v). Addition of peptide is indicated by the arrow.

ratio ($[\theta]_{222}/[\theta]_{215}$) indicates increase of fractional β-structure of the peptide as concentration of water is increased. Addition of methanolic stock into HEPES (final concentration 97.5% water : 2.5% methanol) results in β-structures. Forty-eight hours after mixing, band intensities for the β-like spectrum decrease ~ 40% (data not shown). The half-time for interconversion is clearly greater than 48 h. In order to investigate whether this was due to the formation of large, insoluble aggregates, the sample was spun at 100,000 *g* for 1 h. No changes in absorption spectra were seen between the supernatant so obtained and the starting material, indicating that the aggregates must be small and soluble.

Ac-YC(Acm)-FALQFAPSLITFTVFLLTLW (AcPCH3) is intrinsically fluorescent with a tyrosine at its N-terminus and a tryptophan at its C-terminus. We have also prepared a dansylated derivative with the dansyl group attached to its N-terminus (PCH3-Dans). Excitation of tryptophan at 285 nm leads to either emission from tryptophan or resonance transfer of energy to the dansyl group and subsequent emission from that chromophore. The efficiency of this phenomenon falls off with the sixth power of distance and is 50% efficient at a distance of 23.0 Å (R_0) for the Trp–Dans pair[13,14].

$$R = R_0(E^{-1} - 1)^{1/6}, \tag{1}$$

$$E = \text{Transfer efficiency} = (1 - F_{DA}/F_D), \tag{2}$$

where F_D and F_{DA} are the emission intensities of

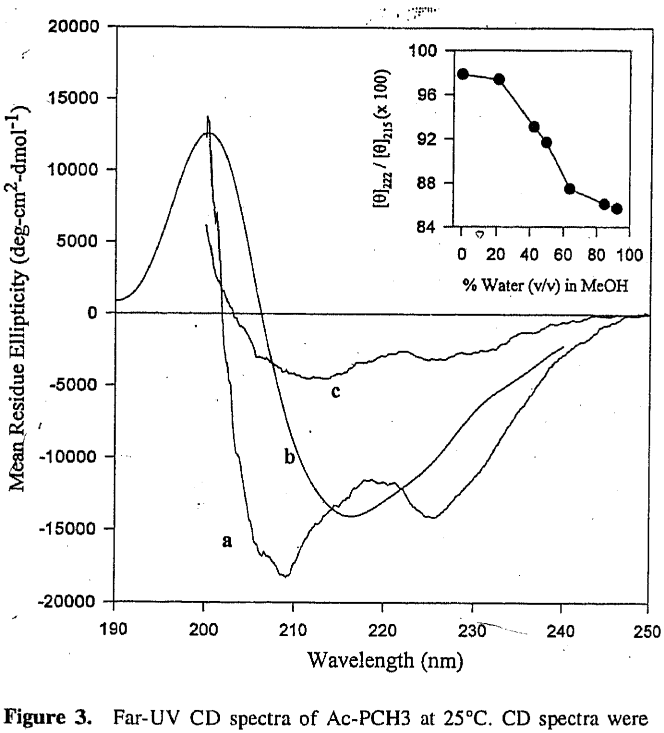

Figure 3. Far-UV CD spectra of Ac-PCH3 at 25°C. CD spectra were recorded on a Jasco720A spectropolarimeter after the instrument was calibrated with (+)-10-camphorsulfonic acid. 1 cm pathlength cells were used and spectra scanned at 50 nm/min in steps of 0.025 nm. 32–64 scans were averaged for each spectrum. Band intensities are represented as mean residue ellipticity ($[\theta]_M$). ***a***, 50 μl methanolic solution of the peptide added into 1.95 ml methanol; ***b***, 50 μl methanolic solution added into 1.95 ml 1 mM HEPES buffer (pH 7.0); ***c***, 50 μl methanolic solution added into 1.95 ml 1 mM HEPES buffer (pH 7.0) in presence of DMPC liposomes. Small unilamellar vesicles (SUV) were prepared following the procedure described in ref. 19 except that DMPC was used. *Inset*: $[\theta]_{222}/[\theta]_{215}$ is plotted against percentage of water in the mixed solvent system.

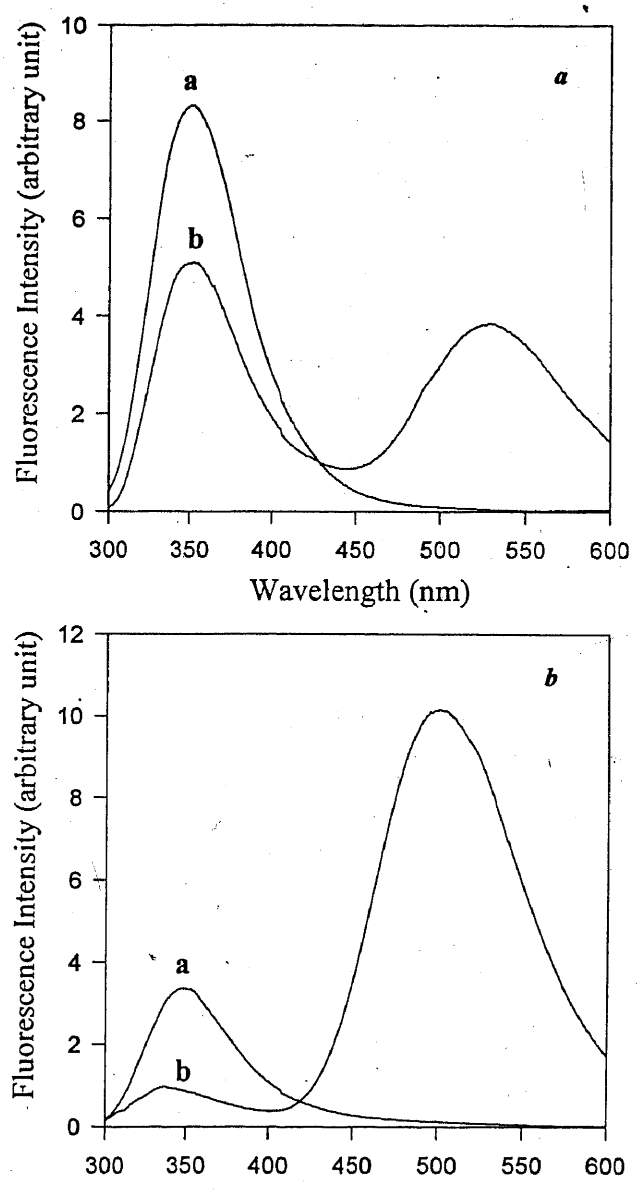

Figure 4. Fluorescence spectra were recorded on a Spex Fluorolog 2 with 1.8 nm excitation bandpass and 1.25 nm emission bandpass with a step size of 0.5 nm per point and an integration time of 1.0 s. Four scans were averaged for each spectrum. All spectra were recorded at 25°C, λ_{ex} = 285 nm and peptide concentration 3.75 μM. ***a***, Fluorescence emission spectra of AcPCH3 (a) and PCH3-Dans (b) in methanol. ***b***, Fluorescence emission spectra of AcPCH3 (a) and PCH3-Dans (b) in HEPES buffer (pH 7.0).

tryptophan in absence and presence of the acceptor respectively.

In methanolic solution (Figure 4 *a*), the presence of the dansyl group decreases the emission intensity of tryptophan by 39% for a peptide concentration of 3.75 µM with a concomitant increase in emission at the dansyl wavelengths. The distance corresponding to this efficiency of energy transfer is 24.8 Å which is less than the 33 Å predicted for a 22-residue α-helical peptide. If aggregated, interchromophore distances are likely to be equal to or less than 20 Å in helical, antiparallel aggregates. The dansyl emission at 530 nm indicates that the dansyl group is not shielded[13], which in turn is indicative that the peptide is not aggregated. We therefore interpret this data as arising from largely monomeric and helical peptides with an average end to end distance of 24.8 Å.

The energy transfer efficiency in HEPES buffer (Figure 4 *b*) at peptide concentration 3.75 µM is much higher (74%) indicative of an average interchromophore spacing of 19 Å. The dansyl emission at 500 nm clearly indicates that the peptide is aggregated[15]. The CD spectrum of the peptide under these conditions shows that the peptide is β-structured. We therefore conclude that the peptide forms stacked anti-parallel β-sheet structures in water. Energy transfer in liposomes is also high, close to 75% indicative of association of the peptide in the membrane phase (data not shown).

The conformation of AcPCH3 is dependent on its environment. Such solvent dependent as well as other types of conformational switching have been reported elsewhere[16–18]. In case of AcPCH3, addition of largely helical peptide from a methanolic stock to a liposome suspension results in largely helical peptide. This is likely to be due to insertion into the liposomal bilayer as the peptide in the suspending buffer is expected to take up β-structures (Figure 3) under these circumstances. The peptide is largely helical in the membranes (Figure 3) suggesting that channels made by AcPCH3 are made up of transmembrane helices.

In summary, the peptide AcPCH3 is α-helical in methanol and in membranes, and takes up β-structures when diluted into water from a methanolic stock. It mediates ion transport through liposomal membranes when a significant fraction of the peptide is helical. This sequence may provide a useful model for studying conformational switching in peptides.

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Predatory efficiency of larvivorous fish, *Gambusia affinis* on the mosquito larvae of *Aedes aegypti* and *Anopheles stephensi*

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The fish *Gambusia affinis* is the best predator on the larvae of *Aedes aegypti* and *Anopheles stephensi*. Predatory efficiency was found maximum on III instar larvae. 2–3 cm group of the female fish performed the best predation. An increase in the larval density accelerated the predatory efficiency in all length groups of the fish. Deprivation of food had an initial stimulation on the predatory efficiency of 2–3 cm group fish.

MOSQUITOES constitute the most important single family of insects from the standpoint of human health. They constitute a far greater threat to man because mosquitoes are the major vectors of a number of pathogens among which malarial parasite deserves attention[1].

There was no aspect of life in the country which was not affected either directly or indirectly by malaria. The magnitude of the problem was great. The economic loss due to the man days lost at one time (1935) was estimated to be nearly Rs 10,000 million/year (ref. 2).

The control of malaria requires more potent insecticides. At the same time massive quantities of insecticides used for the control of malaria year after year started to attract public criticism[3].

Pesticides are potential sources of environmental pollution. The continued use of some long-lived insecticides would lead to world-wide environmental contamination that would be a serious hazard to all animal life, including humans. Thus, a new look at the entire approach to insect control was needed. In order to develop an alternative strategy, the biological control of mosquito was evolved[3].

Larvivorous value of certain fish and their role in mosquito control and anti-malaria programmes are very important[4]. Job[5] reported a list of larvivorous fish of India and the observations on the relative utility of these fish. Reddy and Pandian[6], Menon and Rajagopalan[7] also worked out the larvivorous value of some indigenous fish. De Bach[8] reported a number of factors that influence its success or failure.

Biological control is concerned with the environmental factors affecting the regulation and stabilization of population of insects[9]. Now it is recognized as a potent tool for check on insect population without any harmful effect on the human system[10].

Sharma[3] reported that fish are highly successful in the control of mosquito breeding at low cost and without any adverse impact on environment such as are faced with the application of insecticides, but require further studies on application in the control of mosquito.

A perusal of the literature indicates that little is known of the predatory efficiency of *Gambusia affinis* which is an efficient larvivorous fish. This mosquito fish was therefore selected for the present investigation.

The materials employed in the present study were mosquito fish of the species *Gambusia affinis* (Family: Poecilioidae). Fish were collected from the hatchery maintained by Vellore Municipality Commissioner's office. Fish were reared in 60.75 m capacity hatchery in illuminated conditions[11]. They were sorted into four length groups based on their standard length. The four groups are < 1 cm, 1–2 cm, 2–3 cm and 3–4 cm. Each group was reared in a separate tank.

In insectary, mosquitoes were propagated by Barraud cage device. Adult male and female mosquitoes were introduced in the ratio of about 10 : 2. To maintain this mosquito colony, the adult females were given blood meals at intervals of about 2–3 days by rabbit, and males were fed with 5–10% sugar solution[12].

Fish of each group were introduced into different glass troughs containing separately, I, II, III and IV instars of larvae of *Aedes aegypti* and *Anopheles stephensi*. The number of larvae consumed during 24 h by each was taken into account to determine its predatory efficiency. The impact of length groups of fish on predatory efficiency was determined. The influence of larval density on predation was also resoluted by using III instar larvae in different length groups of fish (III instar showed greater affinity over other larval instars).

The efficient female fish of 2–3 cm length group was taken for the food deprivation study (for 24 h) and its effect on predation was also recorded.

Female *Gambusia affinis* preferred III instar larvae over that of the other instars of *Aedes aegypti* and *Anopheles stephensi*.

The average of III instar larvae of *Aedes aegypti* predated upon, amounted to 70.5/day whereas I, II and IV instars were reduced to 50, 63.5 and 54.5/day respectively. The average predatory efficiency of the entire population of *Gambusia affinis* was calculated as 59.5/day. The greater predatory efficiency was noted for the 2–3 cm group over *Aedes aegypti* and it was 79.5/day. The efficiency of 1–2 cm and 3–4 cm group was 62/day and 46.5/day respectively. The least efficiency was noted in < 1 cm group, i.e. 50.5/day (Table 1).

The average of III instar larvae of *Anopheles stephensi*

Table 1. Predatory efficiency of female fish *Gambusia affinis* over larvae of *Aedes aegypti*. Values (% of larvae consumed) shown are mean of 4 replicates (period 24 h)

Length group of *Gambusia affinis*	Life stages of prey (*Aedes aegypti*) % consumed at density 150 nos.			
	I instar	II instar	III instar	IV instar
< 1 cm	44	52	60	46
1–2 cm	52	66	72	58
2–3 cm	64	86	98	70
3–4 cm	40	50	52	44

Table 2. Predatory efficiency of female fish *Gambusia affinis* against larvae of *Anopheles stephensi*. Values (% of larvae consumed) shown are mean of 4 replicates (period 24 h)

Length group of *Gambusia affinis*	Life stages of prey (*Anopheles stephensi*) % consumed at density 100 nos.			
	I instar	II instar	III instar	IV instar
< 1 cm	19	20	22	18
1–2 cm	21	32	39	28
2–3 cm	33	47	55	40
3–4 cm	24	41	43	37

Table 3. Predation as a function of larval density and predator size. Values (% of larvae consumed) shown are mean of 4 replicates of *Aedes aegypti* and *Anopheles stephensi*

Length group of *Gambusia affinis*	Species name of the prey	Density of III instar larvae			
		50 nos.	100 nos.	150 nos.	200 nos.
< 1 cm	*Aedes aegypti*	44	57	61	75
1–2 cm		56	63	73	89
2–3 cm		77	82	98	99
3–4 cm		33	46	52	64
< 1 cm	*Anopheles stephensi*	17	22	31	46
1–2 cm		23	39	53	61
2–3 cm		46	55	68	77
3–4 cm		40	43	59	63

Table 4. Effect of food deprivation on predation of 2–3 cm length group of female fish *Gambusia affinis* over III instar larvae of *Aedes aegypti*

Time intervals (h)	Larvae consumed (*n*)
1–3	15
3–5	17
5–7	20
7–9	22
9–11	30
11–13	18
13–15	11
15–17	10
17–19	6
19–21	2
21–24	–

predated upon, amounted to 39.75/day whereas I, II and IV instars were reduced to 24.25, 35 and 30.75/day respectively. The average predatory efficiency of the entire population was calculated as 32.43/day. 2–3 cm group also showed greater efficiency against *Anopheles stephensi* (43.75/day). The efficiency of 1–2 cm and 3–4 cm group was 30/day and 36.25/day respectively. The least efficiency was noted in < 1 cm group, i.e. 19.75/day (Table 2).

From this investigation it was inferred that 2–3 cm group female fish shows greater predatory efficiency, and this may be due to their metabolically active state.

The effect of larval density on predation was also studied with III instar larvae of *Aedes aegypti* and *Anopheles stephensi*. The highest predation (81.75 and 61.75/day) was noted when the larvae were stocked in 200 numbers steadily. This observation agrees with Job's[5] and Inasu's[4] findings that the increased larval density induces the predator to kill the prey indiscriminately (Table 3).

Deprivation of food for 24 h altered the predatory efficiency of the predator, female *Gambusia affinis* of 2–3 cm group. The efficiency raised from 15 larvae/2 h to an optimum 30 larvae/2 h; then it decreased to a value of 2 larvae/2 h (Table 4).

The present observation shows that female *Gambusia affinis* is the best larvivorous fish that can be selected for the biological control of mosquito. The highest predatory efficiency was noted in 2–3 cm length group of fish against *Aedes aegypti*. This group had the average consumption of 79.5/day. Its predatory efficiency was increased at the fastest rate when the larval density increased.

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Identification of Sandur schist belt as a potential gold field

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Preliminary mineralogical and geochemical studies with a new approach over the Sandur schist belt indicate a strong possibility of this region becoming a new gold field of India. Gold concentrations ranging from trace to 2.9 gpt have been found at ten new different localities. Earlier workers have reported old workings from Lingadahalli only. Our studies have shown high gold values in the weathered surface rock samples of (i) banded iron formations of Taranagar, Joga and Vibhutigudda, (ii) quartz veins and volcanic rock samples of Papinayakanahalli and Sujigudda–Murutalegudda, and (iii) graywackes of Vibhutigudda. Traces are found in the graywackes of Deogiri also. Most of this gold is invisible and refractory. Detailed geochemical exploration at close interval has been taken up to estimate the economic potential of these deposits. The results of this study emphasize that adoption of new concept-based exploration methodology, in addition to the existing one, can go a long way for a better evaluation of the country's precious metal resources.

SANDUR schist belt (Figure 1) is made up of 2.6 Ga old volcanosedimentary sequences which have been laid down on a passive margin fore land basin and fore arc active basin, later juxtaposed and brought to its present geometry by convergent margin and thrust thickening process[1]. The belt is divided into eight blocks exhibiting lithological, structural and metamorphic discontinuities[1,2]. The structural and stratigraphic interpretations are debated[1–5]. Since this communication is addressed to the gold potential of the belt, these debates are not discussed here. Gold potential of this belt was realized and reported by us[6] and a project was submitted to DST in collaboration with HGML[7]. Subsequently, high Au potential of the belt has been suggested by other authors also[3,8]. Here we report the preliminary results of the work carried out during the last two years. Gold mineralization in the volcanic rocks of the Lingadahalli is known since long and has been reported earlier[3,8–10]. However, other localities (Figure 1) reported here are new finds.

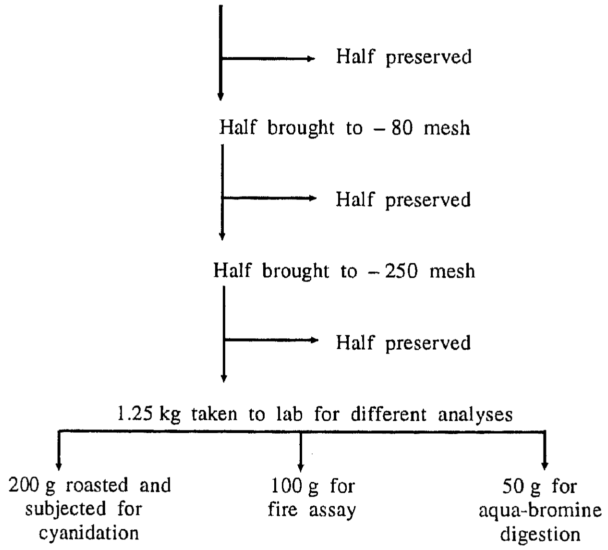
One of the major tasks for gold exploration is to identify new areas in greenstone belts where zones of anomalous gold are present. Such areas have been identified on the basis of a 14-point criterion. We propose that the region to be taken up for first-order bed rock bulk sampling should be tested for (1) presence of thick sequence of basic volcanic rocks, (2) sulphidic BIFs, (3) carbon phyllites, (4) emplacement of dykes, (5) greenschist to amphibolite facies metamorphism (200–400°C), (6) existence of acid volcanics along with metabasalts, (7) generation of fracture and shear system soon after peak metamorphism, (8) multiple ductile wet deformation, (9) emplacement of quartz veins across the schistosity, (10) presence of sulphides in quartz, (11) alteration haloes around quartz veins, (12) visible evidence of carbonatization, sericitization and chloritization, (13) multiple felsic intrusive activity, (14) presence of old workings.

All these characteristics are found in Sandur schist belt and thus we believe that this is one of the main areas which has been subjected to convergent margin processes, generation of secondary hydrothermal fluids, resulting in mineable gold mineralization along certain suitable zones. Along such zones where 10 or more characteristics mentioned above are found, first-order bed rock bulk sampling has been carried out.

Obtaining a representative sample and the procedure for its analysis are the two most important aspects for realistic estimation of the gold content of a prospect. Distribution of gold is inheritantly inhomogeneous. Size of the sample collected is the most important factor to avoid the nugget effect. Small samples generally lead either to over or underestimation of gold content of a given area. Therefore, the bulk sampling and analysis of a large amount of sample is essential to obtain reliable values. Since our petrographic examination under EPMA has shown that gold occurs in submicron size, we collected 10–15 kg sample from different localities and subjected them to sample reduction and analysis, as given in the flow chart below.

Procedure followed for the bed rock bulk sampling and analysis

10–15 kg sample at 50–100 m interval broken by hand and jaw crusher to 1–2 cm chips and divided into two equal parts by quartering and conning.



Half preserved

Half brought to – 80 mesh

Half preserved

Half brought to – 250 mesh

Half preserved

1.25 kg taken to lab for different analyses

200 g roasted and subjected for cyanidation

100 g for fire assay

50 g for aqua-bromine digestion

[§] G.R. Group – represents the entire team of gold project from NGRI and HGML.

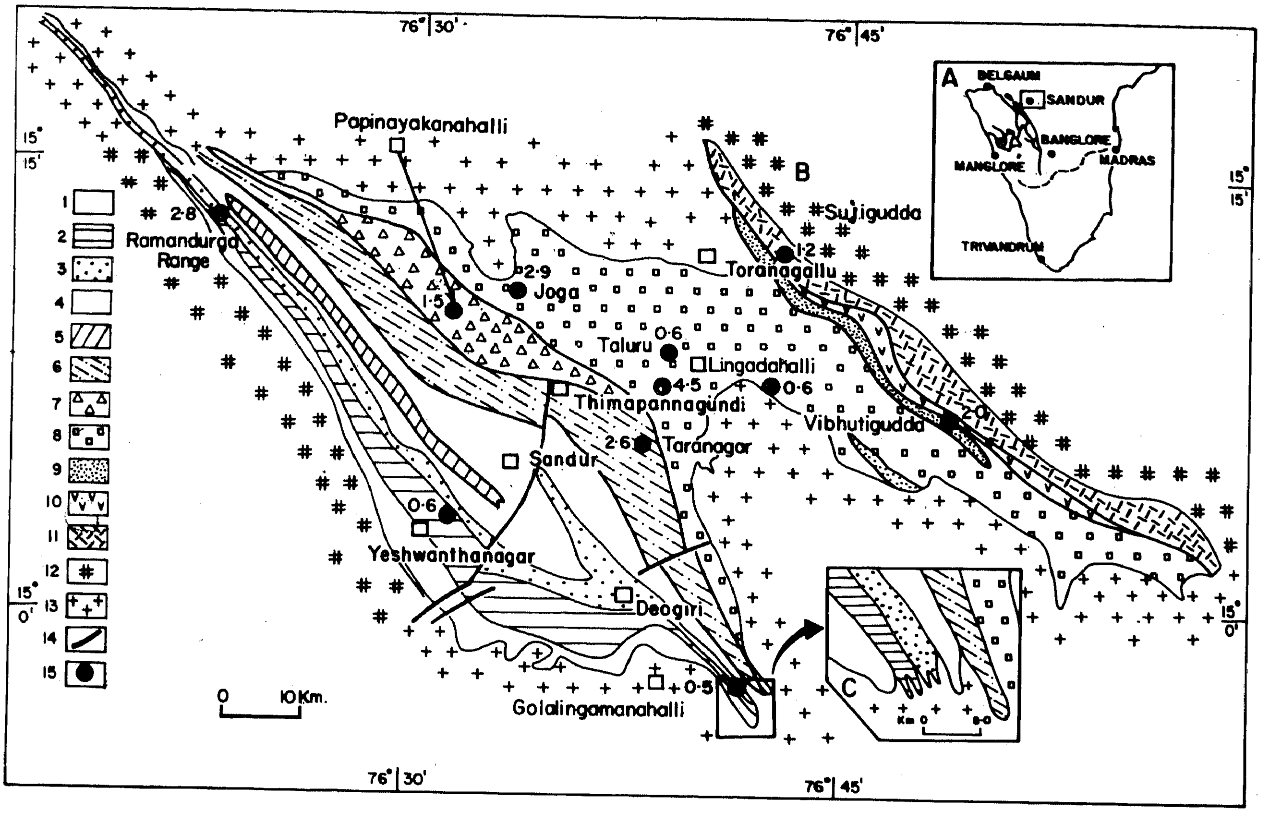


Figure 1. Geological map of Sandur schist belt showing the subdivision into different lithotectonic blocks and the accretion planes which are termed as layer parallel faults (LPF). 1. Yeswanthanagar volcanic block; 2. Deogiri block; 3. Western volcanic block (BIF block); 4. Central volcanic block; 5. Graywackes in central volcanic block; 6. Eastern volcanic (BIF) block; 7. North central acid volcanic block (graywacke-conglomerate); 8. Sultanpura volcanic block; 9. Metasediments of eastern acid volcanic block; 10. Acid volcanic rock; 11. Amphibolites; 12. Granitic gneiss; 13. Granites; 14. Faults; 15. Location of the areas studied for gold exploration.

Cyanidation of 200 g powder (– 250 mesh) for 24 h in the presence of H_2O_2 in an alkaline medium has yielded best results. Samples that yielded gold values of 0.5 gpt or more were analysed by fire assay at HGML and MSPL. Furthermore, these samples were also analysed by ICP-MS and AAS. Details of the analytical procedures developed and adopted at NGRI have been recently published[11]. Values obtained by cyanidation under air agitation are minimum compared to those obtained by aqua-bromine digestion of 50 g powder and analysis on ICP-MS. Cyanidation values compare well with the fire assay and cyanidation carried out at HGML and MSPL labs. An inhouse standard AGI has been made from the sample of gold ore of Ajjanahalli deposit. One 200 g aliquot of the AG-1 is run most of the time along with unknown samples. This standard has given a repeated value of 6.1 gpt Au. This indicates that the rapid cyanidation method can provide the trend of gold anomalies and lead us to narrowing down the area to take up further detailed work in small areas.

Cherty banded iron formation (CBIF) immediately west of Taranagar (Figure 1) is sulphidic and carbonaceous. This band is 50 m wide and 7000 m long in NE–SW direction with 80° dip towards north east. The band is highly fractured, brecciated and such planes are filled with sulphides (Figure 2). It rests on metabasalts and is overlain by a 30 m thick carbonaceous phyllite. Sulphides and oxides of iron and chert are the main constituents. Gold is present in invisible form in sulphides and altered sulphides. Both silver and arsenic are associated with Au. One hundred and ten bulk surface samples were analysed from this zone by cyanidation method, and gold specks thus obtained were weighed on a microbalance. Most of them contained gold trace amounts to 2.6 gpt. Large number of samples gave values ranging from 0.5 to 2.6 gpt. Floatation separates of sulphides yielding 6.0 gpt gold.

The most important locality of a possible mineable gold deposit is around/near Joga (Figure 1), where five sulphidic BIF bands having E–W strike with a combined strike length of 20,000 m, are found. Each band is 40–50 m thick (Figure 3). Fifty bulk samples were drawn and analysed from these bands. Gold content of these bands varies from 0.3 to 2.9 gpt. These bands are hosted in metavolcanics which are interpreted to be Archaean oceanic ridge basalts[1]. These metabasalts are classified as Sultanpura volcanic block[1] and Taluru Formation[3]. The triple junctions of the pillows near sulphidic bands are also sulphidic and contain more than 0.6 gpt gold. At the surface most of the sulphides are oxidized and sulphur smell emanates at the freshly blasted outcrops (Figure 3). Thin argillaceous carbonate bands, probable equivalents of pelagic sediments are also associated. Gabbro, metapyroxenites and doleritic dykes are numerous and in close association. The entire sequence may be a relict Archaean oceanic ridge assemblage and a pseudoophiolite. The sulphidic deposits in these oceanic basalts most probably are partial analogue of modern ridge smoker deposits where high concentrations of gold is found[12]. Aplite, pegmatite and quartz veins of 0.5 to 1 m wide cut across these rocks near the granitic contact and those do not contain gold.

Micron size gold in these bands is locked in sulphides. Best values are obtained after roasting the – 250 mesh powder, which oxidizes the sulphur and releases gold, thus becoming amenable for cyanidation. Detailed sampling and analysis of this zone is in progress. Initial experiments show that with depth the grade of the ore may increase. Since these bands form hillocks, 30–60 m deep open cast mining is easily (spot 3) possible.

Oxide facies BIFs of the southern part of the belt near Golalingamanahalli also contain gold. The values obtained are not consistent. Further work is being carried out to examine the economic viability of this spot. Ramandurga range (Figure 1) in the western part of the belt[13] also contains gold, varying from traces to 2.8 gpt. The high Au content is found in the dolerite dyke and the sulphide-bearing quartz veins cutting across the oxide facies BIFs. However, most of the samples of oxide facies BIFs analysed so far have less than 0.2 gpt gold.

Sulphidic BIFs of the copper mountain also contain 0.1 to 2.0 gpt Au (Figure 1). Radhakrishna[8] and Vasudev[9] have reported old workings and traces to 1.2 gpt gold in the sulphidic ferruginous chert of this hill range.

Gold mineralization is associated with the network of quartz veins in the metagraywackes of the Deogiri and eastern acid volcanic block[1] (Figure 1). Four bulk samples were drawn from the graywackes of the Deogiri block which yielded between 0.1 and 0.6 gpt Au. This area needs more investigations as the deformation pattern of the metabasalts and graywackes shows generation and migration of considerable amount of fluids. On the other side, the graywackes of the eastern acid volcanic block[1] or the Vibhutigudda Formation[3], contain extensive blue quartz veins, with which pyrite, arsenopyrite and chalcopyrite are essentially associated (Figure 4). Forty-two bulk samples (more than 50 kg each) were first drawn from outcrops (Figure 1). These metagraywackes were found to be very promising as most of the samples gave positive results of Au content ranging from 0.1 to 2.0 gpt. The zone sampled is more than 100 m wide and 3000 m long. Detail sampling around the locations, giving more than 0.5 gpt Au will be carried out during coming field season. Radhakrishna[8] has also mentioned the occurrence of gold in the cherts of this region. This entire zone also appears to be suitable for open cast mining up to a depth of 60 m as it forms prominent hill range.

The volcanic rocks of the NCAVB[1] south of Papinayakanahalli (Figure 1), the conglomerate–graywacke bearing part which has been included in the Donimalai Formation[3], also has 40 m wide and 6000 m long acid-intermediate flow which contains 0.9 to 1.5 gpt gold in its surface samples. Ten samples were recently drawn from this flow and all of them gave positive values by cyanidation, fire assay and aquaregia solutions run on ICP-MS. The results from these three analytical procedures were within 10% error bar. Acid volcanic rocks and the blue quartz veins between Murutalegudda and Sujigudda (Figure 1) having abundant box work, after the alteration, and leaching of sulphides, contain 0.8 to 1.2 gpt gold. A thick zone of carbon phyllite and sulphidic BIF is associated with acid volcanics in this zone.

In addition to these locations where encouraging values

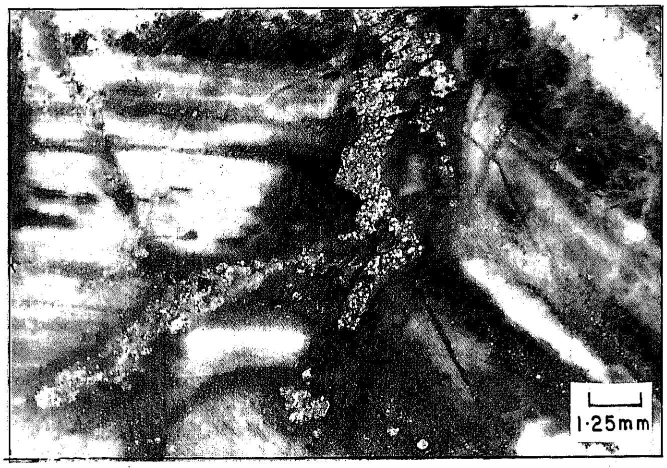


Figure 2. Sample showing the brecciated and fractured nature of the BIFs. Remobilization and deposition of sulphides along the fracture planes is visible.

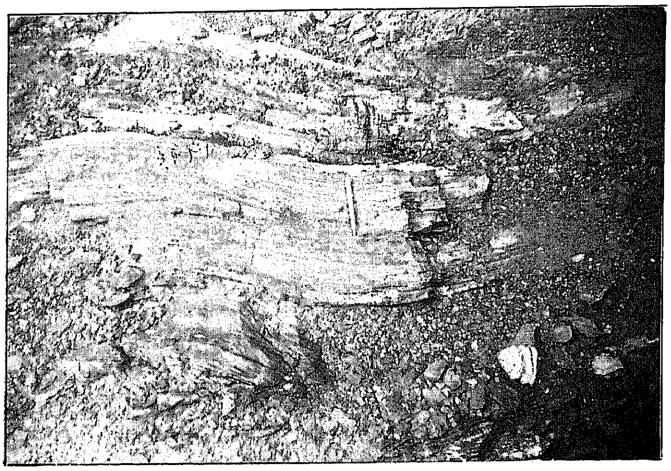


Figure 3. Field photograph showing the sulphidic bands of Joga auriferous BIF.

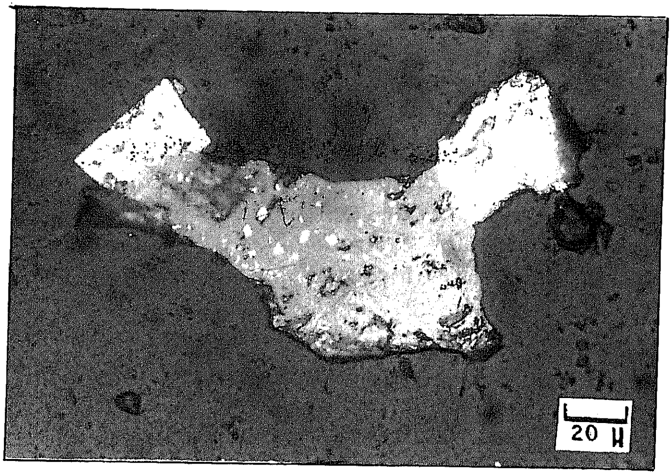


Figure 4. Microphotograph showing the invisible gold-bearing pyrite, arsenopyrite and chalcopyrite in Vibhutigudda.

have been obtained, there are zones such as southeast of Taluru (Figure 1) and southeast of Lingadahalli (Figure 1) where gold values from traces to 0.6 gpt have been found in the random reconnaissance sampling. The Geological Survey of India carried out extensive work around Lingadahalli old workings and reported gold content ranging from 2.5 to 4.5 gpt in acid volcanics and metabasalts[8].

Most of India's gold exploration programme has been revolving around the old workings, which are located on visible gold occurrences. Estimates of the reserves are made through shallow drillings[8]. We have been searching for the elusive Kolar's and Hutti's, which once produced 50 gpt Au[14]. It may be pointed out that only 3 to 4 ppm (gpt) is being mined from Hutti or 2–3 ppm (gpt) from Ajjanahalli. The values more than 0.5 gpt (ppm) are very significant. Total cost of open cast mining and extraction is covered by 0.6 gpt gold. Therefore, if we can identify zones of 1.5 gpt (ppm) then it becomes an economically viable region. Furthermore, studies at several places have shown that 0.2 gpt (ppm) gold concentration at surface, increases to more than 10 gpt (ppm) at depth. Therefore, these values are highly significant and may lead to some economic deposits. There are 800 values. Gold concentration of more than 10 ppb level is considered to be an anomaly in China, Canada and Australia.

A conceptual change in the approach for the gold exploration is needed. Surface bed rock bulk sampling and rapid analysis to prepare geochemical maps of the promising areas have yielded encouraging results. This procedure is more relevant for invisible refractory low-grade ores. Stream sediment surveys may not be successful due to the disturbance of soil through cultivation and construction of small bunds at each elevation change within short distance. The problem becomes more complicated owing to the micron size of the gold grain locked in sulphides and the secondary nature of the soil in most of the low-lying terrains. Recently, convergent margin and ridge subduction processes, resulting in accretionary prisms, have been recognized for generation of the gold deposits in several areas[1,15–17]. Therefore, we propose that all rock types of a suspected convergent margin, where secondary hydrothermal fluid generation and migration is evident should be surveyed by bulk bed rock sampling. After preparation of a large quantity of representative sample, it should be analysed by a combination of cyanidation, ICP-MS and other techniques. The results obtained represent a general trend of gold distribution in such areas. Sandur schist belt being a product of convergent margin processes superimposed on an earlier extensional setting with its highly evident generation and migration of fluids, is one of the most promising greenstone belts which may be transformed into an open cast mining gold field within a short time.

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Soft-sediment deformation in the Quaternary sediments of the Lower Mahi river basin, Western India

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A variety of soft-sediment deformation structures are documented in the Lower Mahi river basin of Western India. They are slumps and slides, syn-tectonic folds, faulting, slickensides and pseudonodules. Majority of the structures indicate syn-tectonic sedimentation and differential subsidence of the basin. The movements took place along several step faults developed parallel to the East Cambay Basin Margin Fault.

THE soft-sediment deformation is defined as the deformation of unlithified sedimentary strata caused either due to intergranular movements[1] or due to intracrystalline deformation that dominates after lithification. The soft-sediment deformation structures are useful in interpreting the syn-depositional tectonism[2] and in deducing ancient tectonic activity[3]. Deformation mechanism in sediments involves changes of stresses in particulate materials initiated by some external agent, which produce deformation structures of varying structural styles[4]. The soft-sediment deformation structures of Quaternary continental sediments of the Lower Mahi Basin have not been documented. The Lower Mahi Basin (Figure 1 *a*) forms a part of a tectonic graben, known as Cambay graben. The fault-bound Cambay graben (Figure 1 *b*), which opened up during Mesozoic[5], comprises marine and continental sediments of Tertiary and Quaternary periods. On account of the northward push of the Indian plate the Cambay basin has progressively subsided[5]. The generalized tectonic evolution of the Mahi basin is shown in Table 1.

The Quaternary sediments are well exposed in the vertical cliffs lining the banks of the Mahi river draining through the alluvial plains of Gujarat. They are structurally controlled by N–S, NW–SE and NE–SW trending fractures[6]. Pant and Chamyal[6] argued that the composite succession of the sediments exposed along the Mahi river dates back to the Middle Pleistocene. The Mahi

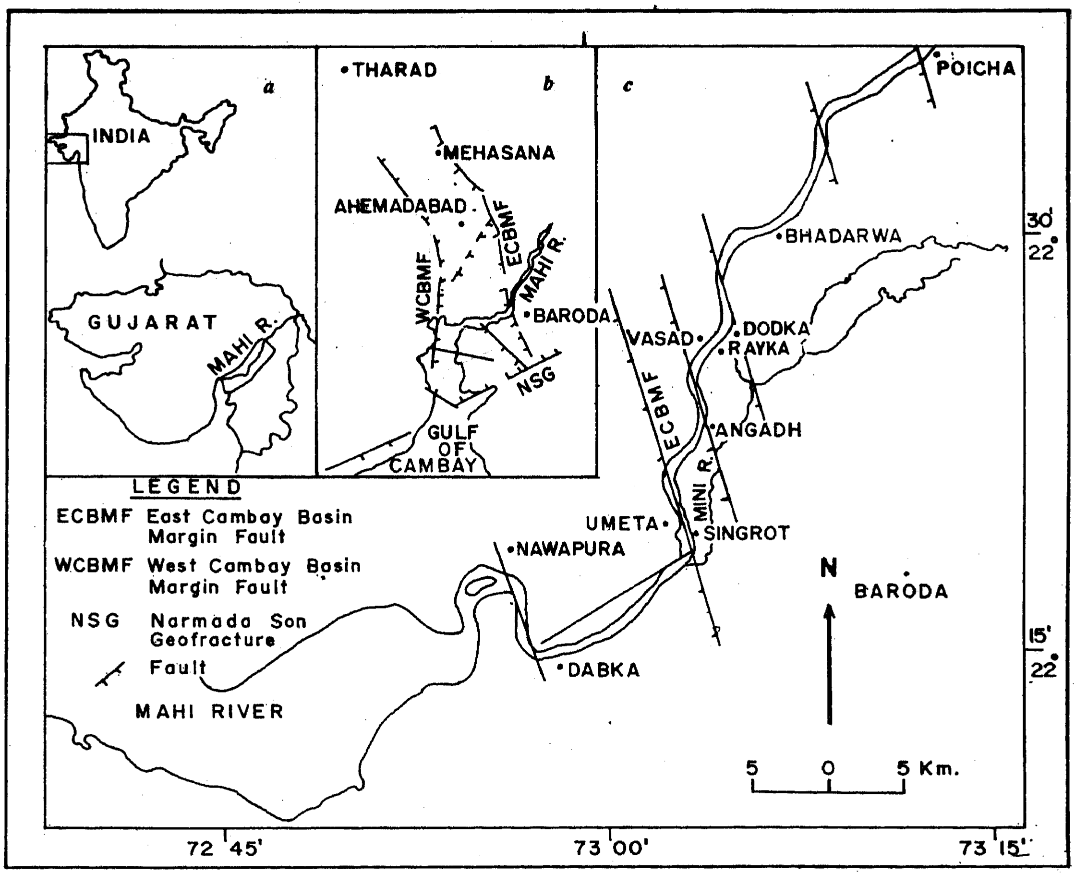

Figure 1. *a*, Location map; *b*, Tectonic map of Cambay Basin based on ONGC data. *c*, Map of Lower Mahi basin showing NNW–SSE trending subsurface faults that controlled the sedimentation. The faults are after ONGC data and MRBC report[15], Govt of Gujarat.

river in Gujarat plains exhibits a general south-oriented meandering pattern, indicating control of subsurface faults, particularly prominent as the river approaches the Eastern Cambay Basin Margin Fault (ECBMF) (Figure 1 *c*). The relation between tectonism, Quaternary sedimentation and the pattern of landscape has been emphasized[7,8].

The lithostratigraphy of the exposed sediments is well established[6,7,9]. The section exposed at Rayka constitutes the most complete section in the entire Mahi valley[10]. The succession comprises fluvial gravels, silts and sands and several paleosols in the upper part (Figure 2). The horizon between gravel-I and gravel-II comprising alternate layers of sands and silts is characterized by the development of some 50 bands of calcrete[10] which are also folded. The sediment column above the gravel-II comprises multiple layers of paleosols, indicating tectonic stability during their deposition. However, the confinement of the deformational structures in the Lower Mahi valley between the two gravel units point to a significant tectonic event of syn-sedimentary subsidence. The deformation structures are confined within the finer-grained sediments between gravel-I and gravel-II.

The fold structures in Lower Mahi are of intraformational character. These in some cases extend up to 15 m and are limited to horizons comprising alternate beds of fluvial silts and sands between the two gravel horizons (Figure 3). Folding of silty beds is particularly prominent. At Rayka the geometric shapes of these folds vary from wide open folds to flexures to monoclines. The calcrete bands show well-developed plunging folds within the finer-grained sediments all along the Lower Mahi river basin (Figure 4). The fold axis trend towards south with a plunge of 10–15°. The wavelength of the folds varies from 1.5 to 2 m with an amplitude of 0.5 m. The monoclines show an apparent steepening towards west, suggesting that the downfaulted blocks lie to the west. The smaller closely spaced minor faults within the strata are significant indicators of syn-depositional tectonism.

Table 1. Generalized tectonic evolution of the Mahi Basin

Tectonic event	Tectonic features	Geologic time
Post-depositional tectonism	Development of NNE–SSW to NNW–SSE fractures related to Cambay rift resulting in the general southward meandering pattern of the river	Late Pleistocene to Holocene
Syn-depositional tectonism	Subsidence of basin as evidenced by syn-tectonic soft-sediment deformation	Lower Pleistocene to Late Pleistocene
Pre-depositional tectonism	Formation of the basin due to reactivation of pre-existing lineaments	Lowermost Pleistocene

The geometry and orientation of these minor fault zones is controlled by the regional fault system. Such minor faults which die out in the overlying horizons have been observed at several locations. These minor faults show a N–S trend corresponding to the major basement faults and exhibit a dominant vertical movement. Each minor fault, exhibits a small increment of slip, the total displacement being distributed on a number of fault planes, particularly in the sandy horizons[11]. A few but ill-preserved slickensides (Figure 5) in clays have also been documented in the Mahi valley which undoubtedly point to a lateral movement. These striations are related to post-depositional faulting. Quite a few slump structures have also been observed concentrated adjacent to the

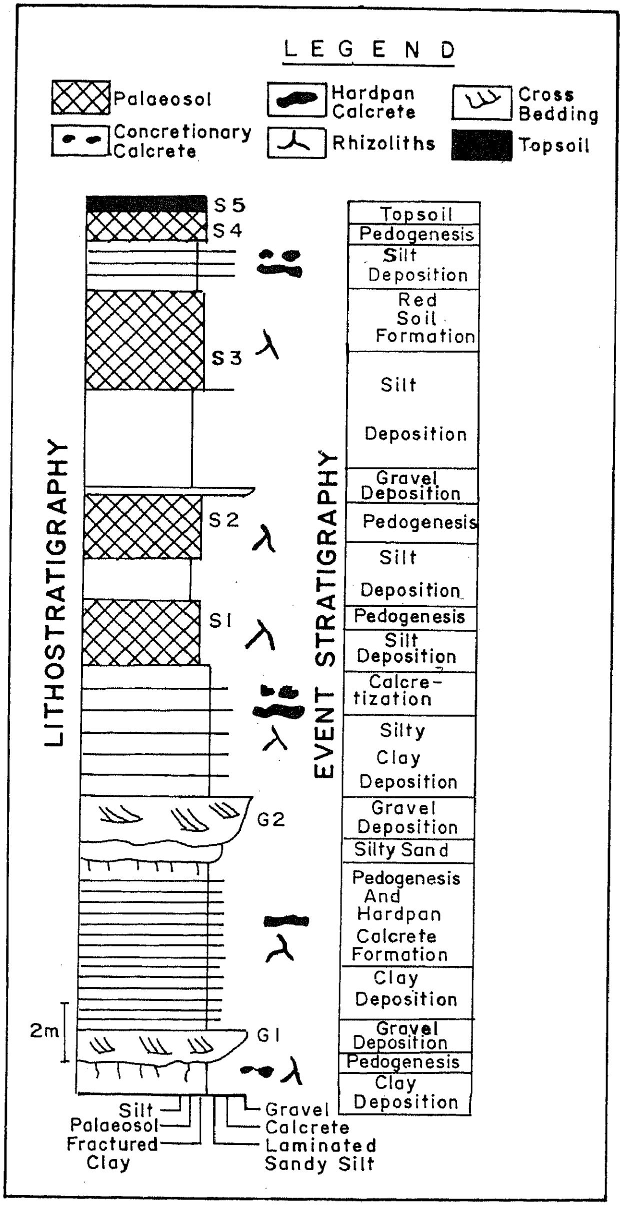

Figure 2. Litholog of the exposed sequence at Rayka[10].

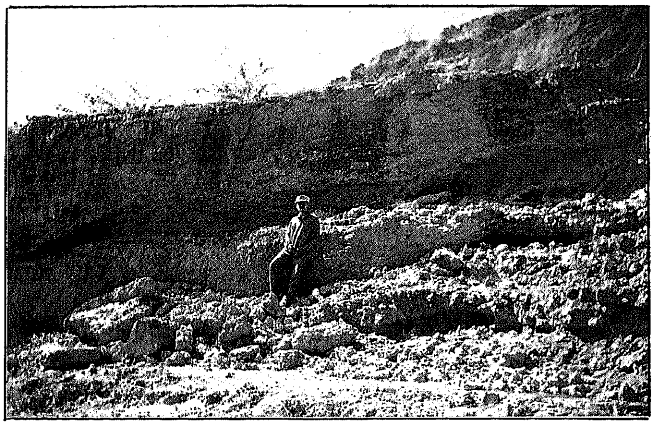

Figure 3. Deformed horizon below the gravel-II at Sili. The folded calcrete bands can be seen prominently.

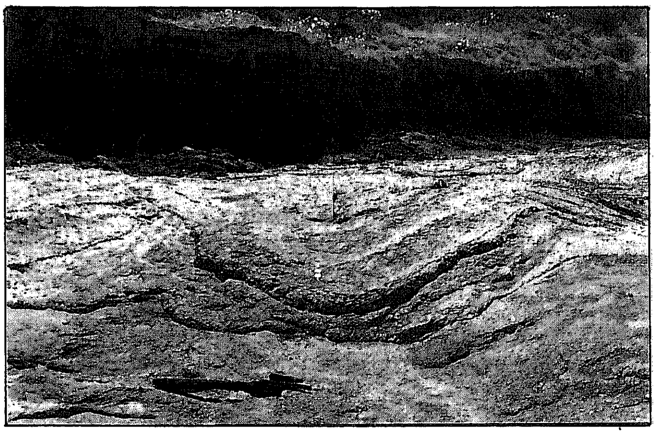

Figure 4. Well-developed southerly plunging (15°) folds in calcretized silty beds at Rayka.

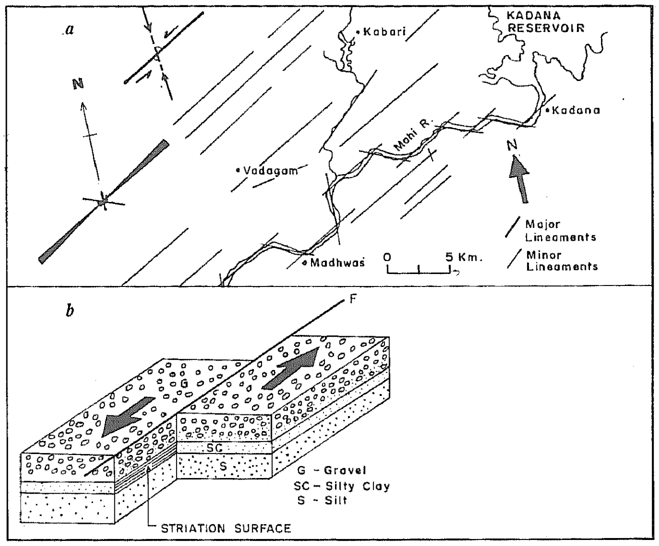

Figure 5. *a*, Map showing NE–SW trending lineaments around Kadana. *b*, Block diagram showing striations on slickensided surface near Kadana indicating post-depositional strike-slip movement.

fault zones, suggesting an unambiguous linkage between the movement along these faults and resulting failure in the sediments. The largest slump structure of syn-sedimentary nature is observed at Rayka. The shear plane of this slump also trends NNW–SSE. Gravity is recognized as an important factor for producing slumps and slides. However, parallelism of the shear planes of the slumps indicate a tectonically induced gravity deformation in the sediments. Pseudonodules give an impression of a horizon of concretion along with ball and pillow structures and are observed at a number of places in the Lower Mahi basin. These are not concretions but are soft-sediment deformation structures[2], formed primarily on account of downslope movement of the interbedded sand and mud due to vertical displacement[13]. Other structures observed, though of less importance are desiccation cracks and disrupted lamination in clays. The empty spaces are now occupied more often than not by veins of calcium carbonate.

The various deformational structures are indicative of syn-depositional subsidence of the basin. Maurya *et al.*[8] emphasized the influence of the continuing subsidence of the Cambay basin on the deposition of the Quaternary sediments in Gujarat alluvial plains. The Lower Mahi basin is characterized by a dominant N–S and NE–SW trending fractures[6]. A series of N–S trending step faults have developed parallel to the Cambay basin margin fault which controlled the sedimentation in the basin[6]. The basalts in the basement east of East Cambay Basin Margin Fault also show step faulting in SW direction[14], conforming with the SW paleocurrent directions[6]. The trends of the deformation structures follow the basement trends. These structures indicate slow subsiding movements along these step faults during the deposition of the Quaternary sediments. The confinement of these structures between the two prominent gravel units points to a significant tectonic episode which ceased after the deposition of gravel-II as indicated by frequent development of paleosols in the upper part of the exposed sequence.

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Oxygenic Photosynthesis: The Light Reactions. Donald Ort and Yocum, C. F., (eds). Series ed. Govindjee. Kluwer Academic Publishers, Netherlands, 1996. Price: Dfl 410, xvi + 682 pp.

This book represents the fourth in the series in *Advances in Photosynthesis* (Series Editor, Govindjee). The first volume, *Molecular Biology of Cyanobacteria* (reviewed in *Curr. Sci.* 1996, **70**, 1101) seems to have received a warm welcome from its readers as this book filled the long-felt void on this subject. The second book in the series, *Anoxygenic Photosynthesis* has not been seen by this reviewer, but he has had a chance to read the third book in the series *Biophysical Techniques in Photosynthesis* which is again a useful addition as it deals with a variety of biophysical techniques from electron microscopy to time-resolved fluorescence spectroscopy; from X-ray diffraction, absorption emission to Mössbauer spectroscopy, neutron scattering, that are currently used for the study of electron and energy transfer processes, for probing the structure of photosynthetic apparatus and mechanism of oxygen evolution. This book provides the state-of-the-art techniques used in the ultrafast processes in photosynthesis.

The volume under review contains topics that constitute the 'heart and soul' of photosynthesis research. The two editors are eminent researchers and they have done a splendid job of collecting excellent chapters from some 58 (most of them internationally known) authors. The editors in their introductory chapter give an overview on the two vital aspects of light reactions in oxygenic photosynthesis: the electron transfer and energy transduction.

All the chapters in the volume have uniform format in presentation and are amply supplemented with cartoons, figures and micrographs that are elegant and illustrative. These chapters not only provide the background in the development of the current state of knowledge on photosynthetic light reactions, but also provide insights into the varied directions in current research in the electron transport and energy transductions processes in oxygenic photosynthetic organisms. Thus this book shall be useful both in teaching of advanced courses in plant and crop sciences and in research programmes. The thirty-two chapters in this volume bring out a comprehensive picture on structural and functional relationships of photosynthetic apparatus in organisms that split water and evolve oxygen. These chapters adequately discuss the components and kinetics of electron transfer by the two photosystems – the components of intersystem electron transport carriers and the structure, assembly and regulation of ATP synthase complex of chloroplasts. The readers would find in this volume new developments in the structure of photosystem I, cytochrome f, light harvesting complexes of plants and algae. One also finds quite attractive discussions on molecular biology of chloroplasts, relating to the regulations at multiple levels in the expression of chloroplast genes; light regulations of nuclear genes required in photosystem light reactions. The readers would also find interesting topics such as evolution of plastids and thylakoid structure; development of thylakoid stackings; photosystem II heterogeneity, trafficking of proteins across the membranes, mechanisms of water oxidation and proton release during water oxidation as well as the exciting developments on structure analysis of reaction-centre single crystals, of photosystem I and on the basic mechanisms of proton electron transfer reactions. All the chapters are presented in a concise, clear and crisp manner. This would be appreciated even by the non-initiated readers. The literature citations are contemporary and comprehensive.

Photosynthesis research expands exponentially and one expects it to continue to do so. Thus, would the book of this type be out-dated soon? We know that the volumes like *Photosynthesis and Related Topics* written by Eugene Rabinowitch and published during 1945–1956 still serve as the source for ideas and outlooks in photosynthesis research. This reviewer, therefore, feels that the current book shall remain as a good source book for teaching and references for many years. Teachers would love to have a copy with them.

Kluwer Academic Publishers seem to have taken the leadership in publishing books, volumes and treatises in plant sciences. The quality of the production of this volume as well as the previous ones is superb and we must thank the series editor Govindjee for his pioneering zeal in bringing out these volumes in a timely manner. For the readers and users of these books, particularly from this part of the globe, the costs are prohibitive. Luckily the paperback covers are available. I suggest that this book must be made available in all college departmental and university libraries.

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Sixty Years of Biology: Essays on Evolution and Development. John Tyler Bonner. Princeton University Press, 41 William Street, Princeton, New Jersey 08540, USA. 1996. Price not known.

I have long been familiar with the terse statement with which a famous man once dismissed a book by another, presumably equally famous, man: 'What is new is not interesting and what is interesting is not new.' Upon reading J. T. Bonner's *Sixty Years of Biology*, one is, unfortunately, left with the same feeling. Unfortunate, because I had approached the book with high expectations: the subtitle of the book is *Essays on Evolution and Development*, and the recent re-emergence of trends toward a synthesis of these two fields is one of the most exciting developments (pun intended) in evolutionary biology. Years ago, G. H. Hardy started off a book by observing that it was a melancholy task for a mathematician to write about mathematics and then created a masterpiece (*A Mathematician's Apology*) that held the reader spellbound. Bonner starts off rather more optimistically, though he does sound a cautionary note in the preface, and then provides the reader with what can only be described as a rather melancholy experience.

The book is organized, to use the word loosely, around four major themes, with a chapter devoted to each. Bonner's brief and sketchy reminiscences on the major events in biology during the past sixty years constitute the final chapter and bring the book to a close. The first chapter, entitled 'Self-Organization and Natural Selection' meanders from D'Arcy Thompson to the girth of trees, via Aristotle, Plato and Protozoa, and leaves the reader with no particular idea of either self-organization or natural selection. Unfortunately, this chapter sets the trend for the rest of the book to follow.

The most interesting chapter in the book is one on gene accumulation and gene silencing. Here Bonner treats phenomena wherein a process that is not originally under genetic control may evolve (even without selection) to become genetically determined. The opposite may also happen: a process genetically determined originally can evolve into a more flexible system that is responsive only to external signals. These ideas are developed further through examples from the study of animal behaviour and development. These are certainly interesting ideas even if the paucity of corroborative evidence renders them somewhat speculative.

The chapters on competition and the division of labour in biological systems contain little that is new to organismal biologists. At the same time, I am doubtful if these would be of much interest to non-biologists because the treatment is often choppy and the author has an annoying tendency to jump from one topic to another rather abruptly. From a scientist of Bonner's stature, I think one is entitled to expect more than a rambling series of musings on rather unconnected topics. Development and Evolution are today rapidly growing areas of biology. In recent years we have learnt a lot about how the development of organisms is controlled and managed by their genes, and about how simple biochemical processes occurring within organisms can give rise to complex patterns. On all of this, the book does not offer the reader anything. On the other side of the coin, evolutionary biologists in the past decade or so have greatly refined their understanding of how development can constrain evolution, and how much of evolution may, in fact, consist in escaping from the shackles of a prescribed ontogeny (e.g. by gene duplication). These aspects of evolution are barely touched upon by Bonner. Indeed, the book really does not do justice to either developmental biology or to evolutionary biology.

All in all, I found the book disappointing. The content is insipid and the penmanship uninspiring. I actually read the book twice, with a four week gap between readings, in the hope of getting some intellectual stimulation from it. Unfortunately, two readings later, I was left with not much more than a greater appreciation of what Mirza Ghalib must have felt when he penned the line '*bahut be-aabru hokar tere kuche se ham niklay*' (dejected did I depart from your neighbourhood).

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Cell and Molecular Biology. S. C. Rastogi. New Age International (P) Ltd. 4835/24, Ansari Road, Daryaganj, New Delhi 110 001. 1996. Rs 550. 502 pp.

Thanks to the emergence of newer techniques of ever-increasing precision at cellular and molecular levels, recent years are witnessing unprecedented rapid developments in the fields of cell and molecular biology. Obviously, the students need to have good resource materials which may provide them a comprehensive view of the current information about the cell and its functioning. As expected, during the past few years many excellent textbooks on cell and molecular biology have become available and several of them have undergone new editions in a rather quick succession. The book under review is an attempt in this direction: as stated in the preface, the author has intended this book to serve as a textbook for undergraduate students.

This book has an impressive get-up, is neatly printed with a large number of illustrations (nearly all in the form of line-drawings) and is relatively (though not absolutely) free of typographical errors. It covers almost the entire spectrum of cell and molecular biology (although defining the limits of this field is nearly impossible today). Each chapter is followed by references and recommended readings and by a number of questions/problems. Thus, on first impression, the book appears useful.

However, when examined in greater depth, many serious weaknesses become apparent. The most glaring is the list of references and suggested readings: nearly all the books and papers, referred to in the book, date earlier than the 1980s with only a very small number of books being as recent as 1985 or so. There is hardly any reference to the very large number of excellent books or reviews that have been published in the past decade. This gives a very wrong signal to young readers as well as to teachers in remote areas who may not have access to current literature. With an ostrich-like view of current literature, it is obvious that the subject matter dealt within the pages of this book is in many cases (though not all) relatively outdated and fails to convey the current excitement in a given area to the students.

Since the book is primarily addressed to under-graduates, it is obvious that none of the topics is dealt in any detail. However, the brief coverage of a topic due to the limited scope, cannot be an excuse for providing outdated and/or misinformed concepts. To substantiate this rather strong statement, I would cite some examples. In reference to ultrastructure of a lampbrush chromosome, it is stated on p. 241 'It consists of alternating regions of high-density granules and low density lateral loops. The high density regions are chromosomes'. The high-density regions within a chromosome cannot be chromosomes! Likewise, the description of polytene chromosomes on p. 242 includes, 'Until recently it was believed that each band represents a single gene with high DNA content. However, analysis has shown that each band on an average consists of about 30,000 base pairs, hence the band may be polygenic and a particular gene may be repeated several times'. There is neither any evidence for a gene being repeated in a band nor are they to be confused with 'polygenes' which is a distinct term in genetics. The exciting area of the immune response is surprisingly included in the chapter on 'Plasma membrane: structure and function'; like all other aspects, this topic is also covered very briefly but what is more worrisome is the fact that the discussion on antibodies does not have any mention of the remarkable processes of gene rearrangements that generate the antibody diversity. The reason given (in the chapter on Recombinant DNA Techniques) for calling the restriction endonucleases as 'restriction endonucleases' is also wrong. Almost all chapters have such confusing, and sometimes erroneous, statements. Obviously, the teacher as well as the student would be utterly confused by such misinformed statements.

As stated in the beginning, the current excitement in biology is essentially due to the emergence of powerful techniques and the seamless amalgamation of cell biological and molecular techniques (facilitated of course by the video and computer applications, etc). While the recombinant DNA techniques are considered in a separate chapter, the microscopic and other cell biological techniques do not find any mention in the book. As a consequence, the cell biological components are rather weak. In fact, the chapters dealing with structures and functions of the various cell components seem to have remained frozen at pre 1980-era!

Writing a textbook is a far more difficult task than writing a reference book. The former calls for a remarkably good comprehension of all the relevant aspects and an equally good control on expression and elucidation of the subject matter. Not all textbooks actually succeed in accomplishing these daunting tasks. The book under review is no exception. Although the author has made an attempt to include the vast area of cell and molecular biology in a compact volume, the many weak points of the book make me hesitant to recommend this book to serious students of the under-graduate classes.

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Architect of India's first test tube baby: Dr Subhas Mukerji (16 January 1931 to 19 July 1981)

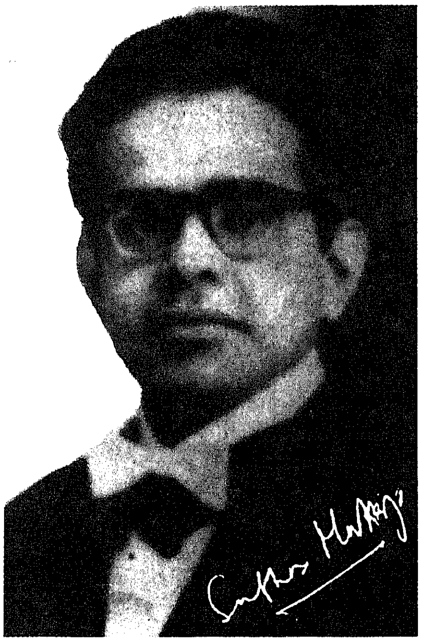
T. C. Anand Kumar

I was recently invited to deliver the Subhas Mukerji Memorial Oration at the Third National Congress on Assisted Reproductive Technology and Advances in Infertility Management held in Calcutta on 8 February 1997. I had never met Subhas Mukerji but I did come to know of his name when it shot into instant fame following a report by K. S. Jayaraman in the *New Scientist*[1] about Mukerji's announcement of the birth of India's first test tube baby on 3 October 1978. Unfortunately, shortly after his announcement, Mukerji put an end to his Life for reasons that were not widely known. The matter was soon forgotten and his claim was generally thought to be one of those scientific aberrations that occur from time to time.

The organizers of the recent Calcutta meeting believed that I was pre-eminently qualified and experienced to delve into whatever material was available regarding Mukerji's past work and throw light on it. The reason for this assumption perhaps lay because of my having played a key role in the birth of another test tube baby, Harsha, on 6 August 1986. This birth was announced by myself, when I was the Director of the ICMR's Institute for Research in Reproduction and Dr G. B. Parulekar, Dean of our collaborating institution, King Edward Memorial (KEM) Hospital, Bombay. Harsha was described as India's first 'scientifically documented' test tube baby because details of Mukerji's work were not then available. I published our technical and procedural details in the *ICMR Buletin*[2]. The work leading to Harsha's birth was executed by a team of scientists from the IRR and clinicians from the KEM Hospital working under my direct guidance and supervision.

Material for this article comprised Mukerji's handwritten laboratory notes, list of his publications and papers presented at various scientific meetings, his correspondence with various scientists abroad and the Government of West Bengal, under which he served and the personal interviews I had with some of major *dramatis personae* in this whole episode.

On going through this material I have no doubt that Mukerji did produce a test tube baby. He and his colleagues were obviously working on their project at the same time as R. G. Edwards and Patrick Steptoe were working in England. These two groups were oblivious of each others work. However, it is interesting to note that Mukerji and Edwards obtained their doctorate from the Edinburgh University and that both had attended the symposium organized in 1971 in Bombay when the Institute for Research in Reproduction was inaugurated. Unlike Edwards and Steptoe, who got kudos for their achievement, Mukerji and his associates were ridiculed by the medical fraternity and victimized by the bureaucracy. Both these humiliating experiences led him to put an end to his life.



Not much of his triumphant achievements nor the tragic circumstances leading to his demise is widely known. There has however, been in existence, as I learnt only quite recently, a small group of scientists in Calcutta who firmly believe that Mukerji did produce India's first test tube baby. This group has perpetuated the memory of Subhas Mukerji for a decade and a half through scientific activities. The sincereity of this group and its strong conviction leaves one without any doubt that Mukerji did not stake a fraudulent claim.

My own research coupled with the existence of this group provide compelling reasons to make known these facts to a larger group of people so that they can appreciate a part of the India's triumphant, and yet tragic, scientific heritage.

Announcement of the birth of India's first test tube baby

Calcutta Doordarshan flashed the news of the birth of India's first and the world's second test tube baby on Tuesday, 3 October 1978 at 11.44 AM. The team responsible for this procreation comprised Subhas Mukerji, Professor of Physiology, Bankura Sammilani Medical College; Sunit Mukerji, Professor of Food Technology, Jadavpur University and Dr Saroj Kanti Bhattacharya, Associate Professor of Gynaecology and Obstetrics at the Calcutta Medical College. This news was widely reported in almost all the leading dailies of Calcutta[1,3–5]. Some essential details of the procedure were described and a photograph of a new born baby was published. The baby girl was given a pseudonym, Durga, after the most popular goddess of Bengalee Hindus. The mother's name was mentioned as Mrs Bela Agarwal[5].

Technical procedure as described by Subhas Mukerji and colleagues

At the behest of the West Bengal Government, Subhas Mukerji, Sunit Mukherjee and S. K. Bhattacharya submitted a signed report on 19 October 1978, sixteen days after Durga was born. This report titled 'Transfer of *in vitro* fertilized frozen-thawed human embryo' briefly describes their work leading to Durga's birth. The contents of this crucial document are not widely known and is therefore reproduced verbatim:

'Mrs Agarwal aged 31, married for 16 years, a case for primary infertility due to diseased tubes had apparently normal ovulatory period on 12–11–77. On 14–11–77, 16–11–77, 18–11–77 and 20–11–77 she had 4 ampoules of inj. Pergonal i.m. twice daily each day; on 22–11–77 she received 6000 I.U. of hCG (Physex, Leo) i.m. On 24–11–77 she was subjected to colopotomy when each of her ovaries were delivered through the colpotomy wound one after the other. Follicles measuring approximately 2 cm were identified and the follicular content aspirated by glass syringe using short bevelled needle. The aspirated material from 5 follicles also containing follicular fluid were collected in separate flat bottomed sterile vials and put into an incubator straight away set at 37°C. Each of these were examined microscopically using ordinary microscope and the corona cell covered preovulatory oocytes were identified subsequently. The incubation was continued for 4 hours approximately. Then husband's semen was collected, washed twice and 2×10^6 spermatozoal suspension per ml was prepared using modified Tyrode's solution with serum. The identified oocytes were transferred into the spermatozoal suspension and incubated for approximately 24 hours. After this the oocytes were transferred to freshly collected nontoxic heparinized cervix-uterine fluid drops containing approximately 100 I.U. Penicillin/ml, under paraffin. The incubation was continued (37°C) for another approximately 72 hours. The oocytes examined microscopically five in number were all cleaving showing 8 or more cleavage cells. The suspended embryos were equilibrated in a stepwise fashion at 20°C with 1.5 M Dimethyl Sulphoxide and frozen in ampoules as follows:

'Based on the available information in the literature this simple technique was adopted to freeze, store and thaw the embryo. Initially the sealed ampoules containing embryos were cooled slowly in alcohol bath in a glass dewar by adding carbon dioxide snow at regular intervals. A blank experiment was done to see that the temperature drops at the rate of about 0.5°C/min. After –7°C the rate was increased to about 1°C/min and the addition of snow was continued for about an hour. Then the ampoules were chilled in liquid nitrogen in a dewar. The ampoules were stored in cryogen for about 53 days.

'Before use the ampoules were thawed individually slowly at the rate of about 4°C/min by adding continuously fresh alcohol to the alcohol bath until about –7°C. Then the ampoules were transferred to iced water and finally warmed with tap water.

'On the day of transfer the embryos after thawing were incubated for at least 1 h at 37°C. Mrs Agarwal had a delayed period on 3–1–78. On 18–1–78, 19–1–78 and 20–1–78, three embryos suspended in cervical fluid were transferred into the uterus near the fundus per Os using a microsyringe attached with a transparent plastic tube. Mrs Agarwal missed her periods with clinical symptoms of pregnancy and immunological test for pregnancy was positive on 14–2–78 and again on 21–2–78. She was delivered of a healthy female baby weighing 3 kg, 350 gm on 3–10–78 by lower segment Caesarean section.'

Subhas Mukerji's academic background

Subhas Mukerji was a medical graduate of the Calcutta University. He went on to work for his D Phil degree under Professor Sachidananda Banerjee of the same University. The subject of his study was biochemical changes in normal and abnormal pregnancy. He was awarded a Colombo Plan scholarship to work in the MRC Clinical Endocrinology Research Unit in Edinburgh under Professor John A. Loraine, an eminent reproductive physiologist. His research was directed to developing new, sensitive bioassays for gonadotropins using ovaries in intact rats. This was one of the hot topics of the sixties when radioimmunoassay had not yet made its advent; one had to depend on these very sensitive, but cumbersome, assay methods to understand the physiology of reproduction. Indeed, some of the very early foundations of reproductive endocrinology were laid using such assays. It must be noted that there were very few laboratories in the world at that time who had the competence to undertake such studies. A person had to be really hardworking, dedicated and putting in long hours of work to get meaningful results out of such assays. I mention all this to highlight the fact that Mukerji had acquired considerable research skills in such areas that are normally seen only in those confined to the research laboratory.

Mukerji's research blossomed when he returned to Calcutta. He used his considerable technical skills, matched with a fertile mind, to study a wide range of reproductive physiology–pathology problems. From the list of Mukerji's publications I could pick out the following interesting lines of research carried out by him:

He was familiar with the pharmacological (mainly gonadotropins) methods of inducing ovulation and spermatogenesis in human subjects.

He was interested in innovative gynaecological surgery.

He also worked on experimental animals and was able to translate these findings to the human situation as exemplified by his work on finding the luteotropic effects of DHEA and testosterone in rats and human beings.

He demonstrated the presence of hCG-like substance in non-pregnant human endometrium both by biochemical isolation as well as by bioassay. He speculated on the possible role of the uterine hCG-like substance on foetal development – an interesting lead that has alas not been followed up.

More basic issues such as the androgenization of the female rat brain and acyclicity of menstrual cycles in women were also investigated by him. His studies showed that acyclicity of reproductive cycles as seen in amenorroheic women and neonatal androgenised rats was not primarily due to changes in the hypothalmo-hypophyseal system but in altered ovarian secretions because unilateral ovariectomy of such rats as well as wedge resection of polycystic ovaries in women restored cyclicity.

Another area that is of profound relevance today is his theory that emotional stress was the cause of polycystic disease. He postulated in 1970 that emotional stress was a contributory factor to the development of Stein–Levanthal syndrome or the polycystic ovarian disease leading to infertility. With the current availability of transvaginal ultrasonography, a significantly larger number of women are now being classified as having polycystic ovaries. Is this a reflection of the advent of the new investigative tool that has become available? or is it a reflection of stress experienced by the modern female urbanite? It is only in recent times that stress has been recognized as a contributory factor to infertility and other reproductive disorders[6].

Mukerji's presentation of his work at scientific meetings

A substantial amount of interest was generated in Mukerji's claim following his announcement over Calcutta Doordarshan. He was invited to present his findings at many scientific meetings. Mukerji has recorded these meetings in an official letter dated 1 December 1978, addressed to the Director of Health Services (DHS), West Bengal Government.

Mukerji presented his findings in the section on 'Ovum implantation' at the V International Congress on Hormonal Steroids held in New Delhi from 29 October to 4 November 1978. His presentation was chaired by B. B. Saxena of the Cornell Medical School and S. K. Manchanda of the Department of Physiology, All India Institute of Medical Sciences, New Delhi. The presentation was actively discussed by some eminent scientists of that time and who had attended the Delhi meeting such as, John Biggers and Kenneth Ryan from Harvard, Mettler from Kiel, and Bettendorf from Hamburg. Immediately after this meeting Mukerji gave a public lecture at the Gangaram Hospital, New Delhi. He was interviewed at great length by B. B. Saxena and G. P. Talwar in a special programme which was telecast by Delhi Doordarshan on 3 November 1978. Mukerji has gone on record of having discussed his findings with some of the leading scientists in reproductive sciences at that time in Delhi: B. K. Anand, G. P. Talwar and Somnath Roy.

Mukerji was invited by J. Nag Choudhary, Professor of Physiology to speak at the Satellite Symposium of the V International Congress on Hormonal Steroids in Varanasi. His talk was chaired by a well-known scientist, Udupa, the then Director of the Department of Surgical Sciences at the Banaras Hindu University.

Mukerji was invited by Gogoi, Professor of Obstetrics and Gynaecology, Gauhati Medical College to speak on his technique of embryo transfer. He was awarded a 'MANPATRA' in recognition of what the Society thought to be an outstanding piece of work.

He was met by Ambassador Marshall Green, Coordinator of Population Affairs, US State Department, Washington on 6 November 1978 and later by Kessel, Population Division, University of North Carolina, USA to discuss the relevance of his work *vis-à-vis* the Population Problem.

He made a major presentation on his work at the Indian Science Congress in 1979 in Hyderabad.

Reaction of the medical fraternity

Tarun Banerjee, a leading gynaecologist of that time in the Calcutta Medical College, is reported to have stated that 'there should be a scientific discussion in detail about three methods, collecting ovum from the mothers ovaries, fertilization of the ovum and its development and pushing it back into the mothers uterus, which were very complex...'. (ref. 4). These are the only published criticism I could get hold of. I understand that similar doubts and skepticism were expressed by several others at that time which vitiated Mukerji's claim.

The brief press reports that appeared along with the announcement of Durga's birth[1,3–5], did address all the three issues raised by Tarun Banerjee but was not fully comprehended by the 'experts' of those times because of limited knowledge in this frontier area of research.

West Bengal Government's reaction to Mukerji's claim

The Government of West Bengal appointed an 'expert committee' under the Indian Medical Association (West Bengal Chapter) and the Bengal Obstetrics and Gynaecology Association to investigate into the claims made by Mukerji and his colleagues in the brief report sent to them on 19 October 1978.

The Committee was headed by a professor of radiophysics and assisted by a gynaecologist, a physiologist and a neurophysiologist. None of these committee members could have had any background or insight into the modern science of Reproductive Technologies, a subject upon which they were to hold an inquiry. One wonders the reasoning of the West Bengal Government for nominating a person from a totally alien field to head this important committee.

This inquiry committee met on the 18 November 1978 to critically review the report given by Mukerji to the DHS (reproduced in an earlier part of this paper) but ended in a denouement of Mukerji's claim. Mukerji wrote to the DHS on 1 December 1978. Mukerji stated that the kind of inquiry instituted required adequate time for him to prepare the report. He had to hurriedly prepare his report for the inquiry committee at short notice (the report was prepared within two weeks of the announcement). With very sound reasoning, he did not reveal all his data because he wanted to 'publish these in recognized scientific journals after the reproducibility of the work is reasonably assured'. He went on to state: 'The final concentration of DMSO used before freezing as well as the exact indigenous method of cooling were deliberately omitted from the report, like (also) the steps for removal of DMSO before thawing. Certain essential intermediate steps, during the whole procedures also involving the use of undisclosed and enriched media were completely omitted. I had to be careful to guard our unpublished data, because by that time I became aware of the penetrating efficiency of the tentacles of the mass media.'

Areas of dominant research in the seventies

Population overgrowth *vis-à-vis* diminishing finite natural resources was the dominant thought of concern. The contraceptive pill and the Intra Utrine Device (IUD) emancipated the coital act from procreation. These discoveries gave impetus to seek out newer contraceptives through basic research. The eradication of small pox from the planet Earth in early seventies through vaccination encouraged the then Health Minister, Dr Karan Singh to believe that a contraceptive vaccine would be the answer to eradicate the bane of overpopulation. Funding for contraceptive research reached its nadir from the Government of India as well as from many international agencies. Major developments in contraceptive research occurred in the All India Institute of Medical Sciences; the Department of Zoology of the Delhi University; the National Institute of Family Planning, New Delhi; the

CDRI, Lucknow; the IRR, Bombay and the Indian Institute of Science, Bangalore. Those were heady days indeed when scientists not only in India but on a global level were vying with one another to be the first to produce a bettter alternative to the Pill and IUDs.

Not much attention was paid during this period to research being made by a handful of scientists around the globe including Subhas Mukerji. Very few people in the developing countries, which were beleaguered with population overgrowth, even considered pro-fertility research of being of any use. Conferences on reproductive sciences in the seventies attracted large number of participants talking about new information on how new contraceptives could be developed. There were hardly any papers on the new reproductive options. Research and development of the new reproductive options took place against such a background – without public interest, without public support and general ignorance for the need to carry out such research. Medically Assisted Reproductive Technologies (MARTs) were not a fashionable subject for research then as it is today.

Calcutta in the seventies

Calcutta has a hoary history of producing some of the brilliant scientists during the end of the last to the beginning of the present millennium. Calcutta lost most of its glory in more ways than one when the Capital was shifted to Delhi. Partitioning of SONAR BANGLA following Independence of India was another human tragedy with the influx of a massive refugee population from neighbouring East Pakistan. This downward trend in Calcutta's glory reached its lowest level in the seventies following the post-war entry of refugees from the newly created Bangladesh. There was serious economic crisis. This was the OH CALCUTTA era. Mukerji carried out his work under such dismal economic conditions. But as the famous saying goes, the lotus grows in a marshy tank, so also Mukerji's work grew under daunting conditions. One cannot but admire his tenacity and perseverance.

It is noteworthy that the seventies witnessed a bleak period in Indian history when a State of National emergency was promulgated and forced sterilizations were the order of the day in the name of family planning. Anyone who was concerned with reproduction research spent all efforts to develop better contraceptives. Those who did not conform to the then National goals were summarily arrested and questions asked later.

Considering these abysmal conditions one can understand why not much attention was being given to the then unfashionable research being carried out by Mukerji and why he was so cautious and secretive about his work.

A critique of Mukerji's technique

The brief description given by Mukerji in his letter dated 19 October 1978 to the Director of Health Services, Government of West Bengal, the reports he gave over the television interviews and reported in the lay press describe how Mukerji carried out the procedure of *in vitro* fertilization.

Collection and evaluation of spermatozoa

Durga's father was found to have a low sperm count[3–5], according to Mukerji who obviously was aware of the diagnostic value of semenograms as evidenced by the papers he presented[7,8] much before the WHO laboratory manual for the examination of human semen came out in 1980. Mukerji also knew that such a condition can be effectively treated with gonadotropins[9]. Gonadotropin therapy is now routinely used to treat men with low sperm counts.

Ovarian stimulation

In his report dated 19–10–1978 to the DHS, Mukerji stated that he had treated Mrs Agarwal with hMG 76 ampoules given twice a day and on alternate days and starting from day 3 to day 9 of the cycle. She was given 6000 I.U. of hCG on day 11 of the cycle and she was subjected to oocyte aspiration some 48 h later. Mukerji was able to aspirate 5 follicles by this method. In today's context this ovarian protocol will not sound outrageous because controlled ovarian hyperstimulation is the standard procedure for all women subjected to IVF. However, until 1980 December 'conventional wisdom' in Britain, Australia and USA dictated that stimulated cycles were unsuitable for oocyte collection and therefore oocyte aspiration was restricted to natural cycles[10]. It was only in 1981 that other scientists resorted to ovarian stimulation. The Australians, who were the next to announce the birth of IVF babies, began to use clomiphene citrate for ovarian stimulation[11] in 1981. The Norfolk group in the USA, who were the third to report the occurrence of an IVF baby, began to use with hMG and hCG in their IVF programme with success[12] in 1982. It is noteworthy that Mukerji was far ahead of his time in successfully using an ovarian stimulation protocol before anyone else in the world had thought of doing so.

Ovum pick-up for IVF was a problem during the early days. The British team had used a laparascope to harvest oocytes. The advent of ultrasonography later on opened out a new avenue to aspirate oocytes transvesicaly under ultrasound guidance by making apercutaneous insertion of a long needle traversing the abdominal wall, the urinary bladder and finally the ovarian follicle. The advent of the transvaginal probe has made it possible to collect oocytes per vaginum and this is the standard procedure used now.

Mukerji's originality was that he was able to access the ovaries by a very simple operation on the wall of the vagina. Stimulated ovaries enlarge and drop down towards the Pouch of Douglas. A small incision on the posterior wall of the vagina would allow the ovaries to fall through this opening. This reminds one of how a biologist gains rapid access to the ovaries of rats by a very simple lateral, abdominal incision. The entire procedure was accomplished within a couple of minutes. Mukerji's research experience with rats and humans had helped him take this very simple and original approach of gaining access to ovaries by posterior colpotomy.

One wonders why this very simple approach did not gain popularity. After all today oocytes are aspirated per vaginum under ultrasound guidance.

In vitro culture techniques

The freshly aspirated oocytes were incubated for 4 hours before inseminating them with the husband's semen that was processed in protein-supplemented Tyrodes solution. This is exactly what is done even to this day in almost all

IVF programmes to accomplish *in vitro* oocyte maturation; processing semen is essential for 'sperm activation'. The oocytes were exposed to processed semen for a period of 24 hours and later incubated for another 72 hours in a mixture of cervical-uterine fluids. The use of such fluid is not described elsewhere. However, the use of a synthetic fluid, similar to that found in the human fallopian tube, has been described to be useful for *in vitro* embryo culture procedures.

The methods of *in vitro* fertilization and embryo growth are described in detail in Mukerji's letter to the DHS dated 19 October 1978 as well as in a publication in an obscure journal[13]. Mukerji stated '. . . It also appears that for cryogenic preservation of embryos with a relatively larger number of blastomeres (more than 8 cells) may be preferable.'

'Few pre-ovulatory human oocytes collected from a married woman by surgery were fertilized with spermatozoa from the husband and cleaved *in vitro* and subsequently frozen slowly to about 196°C after stepwise treatment with dimethyl sulphoxide. One such frozen embryo was subsequently thawed slowly and when transferred into the uterus of the woman apparently resulted in the production of a clinically normal female baby after normal period of gestation.'

Here is clear published evidence of how exactly Mukerji carried out his version of *in vitro* fertilization and embryo transfer.

Cryopreservation of embryos from mice, rabbits, sheep goats and cattle was reported between 1971 and 1979. The first report on the successful cryopreservation of four to eight cell human embryos appeared as late as 1981 and the first successful clinical outcome of the transfer of thawed human embryos was reported in 1983 by Trounson and Mohr[14]. A WHO Report[15] states 'Embryo cryopreservation has now become a routine adjunct to IVF procedures, and various methods of freezing are employed. The method that has yielded the best results in terms of simplicity, efficiency and reproducibility is one that involves freezing of one- to three-day-old embryos (one to eight cells) in a controlled biological chamber that cools the embryos to sub-zero temperatures in the presence of a cryoprotectant 1,2-propanediol. Other cryoprotectants that are used are dimethyl sulphoxide (the same cryoprotectant was used by Mukerji) and glycerol.

It may be noted that Subhas Mukerji reported the successful cryopreservation of a 8-cell embryo, storing it for 53 days, thawing and replacing it into the mother's womb, resulting in a successful and live birth as early as 1978 – a full 5 years before anyone else had done so. This small publication of Mukerji in 1978 clearly shows that Mukerji was on the right line of thinking much before anyone else had demonstrated the successful outcome of a pregnancy following the transfer of a 8-cell frozen-thawed embryo into human subjects transferring 8-cell cryopreserved embryos.

Controversies and debates following birth of test tube babies

Edwards and Steptoe's pioneering work attracted considerable public attention on a global scale. These have been eloquently described in the two books[16,17]. The manner in which the test tube baby project was handled by the Edward–Steptoe team is a classical example of how a combination of transparency, scientific debate as well discussing moral, ethical, legal and religious issues can turn the tide from one of societal hostility to that of societal acceptance. Almost every aspect of Edwards work, starting from the *in vitro* culture of human eggs obtained from ovariectomized patients to *in vitro* fertilization, since the sixties to the late seventies was chronicled in peer-reviewed scientific journals, and debated intensely at public meetings. Edwards is very conscious of moral and ethical issues arising out of the new reproductive technologies. He has been careful to subject every aspect of his human experiments to institutional ethics committees, obtained their permission before actually starting of the experiments even though this meant his losing out on being the 'first ever' on several new technologies such as intrafallopian transfer of gametes and embryo cryopreservation. Perhaps no single individual has done so much for this field than Edwards. He is a trend setter on how research in new areas concerning humanity should be conducted. Today if MARTs is a respectful discipline, the credit should go the Edwards and his team mates.

Our own test tube baby project in Bombay attracted a great deal of attention, including that of the Indian Parliament where a memeber had tabled a question in the house asking for details of this event. Fortunately, I had all the essential scientific documentary clearances and evidence in the form of the Approval of IRR's Scientific Advisory Committee, the Hospital Ethics committee's clearance and finally photographic documentations of the entire procedure. I was able to foresee such events well in advance and be prepared for such an eventuality as had occurred because 'I was able to look further because I could stand on the shoulders of great men before me' – in this case, Subhas Mukerji.

If Subhas Mukerji feared public criticism he was not alone. Edwards and Steptoe narrate how they were spurned by fellow scientists and how they were denied research funds. Zeilmaker in Holland, Howard Jones in the US and Trounson in Australia have all at some time or the other been criticized for their work in *in vitro* fertilization of human eggs. Indeed, Edwards describes in his books[16,17] how he was able to silence the Press and even legally penalize them for misrepresenting his statements. All this makes very interesting reading.

Mukerji driven to put an end to his life

The DHS, in a letter dated 28 December 1978, specifically prescribed Mukerji from attending 'any conference in the future without prior permission from the competent authority'. Mukerji was invited by the Primate Research Centre of the Kyoto University, Japan, on 25 January 1979 to attend a closed meeting at their expense to discuss details of Mukerji's work. Mukerji applied to the DHS for permission, which was promptly denied vide their letter of 16 February 1979. The letter directed Mukerji not to leave the country without prior clearance from the Government. Subhas Mukerji shortly afterwards suffered a heart attack. His request for special leave was declined but his request for transfer was promptly accepted and, at 'pleasure of the Governor of West Bengal' he was transferred to the Regional Institute of Ophthalmology as Professor of electrophysiology on 5 June 1981. The Government preventing him from presenting his work at scientific meetings, denying him leave to write up his results and the humiliation he was subjected to by his colleagues in Calcutta were some of the things that the sensitive Subhas Mukerji could not bear. His transfer to a department in which he had no expertise was the last straw on the proverbial camel's back for Mukerji. This transfer order was dated 5 June 1981. Mukerji gave up fighting the system and ended his life on 19 July 1981, 44 days after the transfer order was issued.

Much of Mukerji's work remained unpublished not because he did not have data but because he was not given a chance to do so by his administrative Ministry in the Government.

Why did Durga and her parents choose anonymity?

There remains the question as to why Durga's parents wished to remain anonymous when most parents of IVF babies all over the world came out with their story of how medical science and technology aided their infirmity. One must understand the Indian psyche which considers barrenness to be a curse. Unmarried girls are considered to be unfortunate and therefore it is the desire of most parents of girl children to find a suitable husband and have them settled in life as soon as possible. Durga's parents echo such sentiments; her mother was married at the age of 16 and could not have a child for next 16 years – almost for the entire period she lived before marriage. In my discussions with them it became obvious that they did not wish to have much publicity as this may adversely affect India's first test tube baby's chances of getting married. I was introduced to Durga by her parents at my request. She is a well-educated young woman and is aware of the origins of her birth. She was all for advancement of science but was not prepared to be viewed as a curious object and subjected to the media intrusion into her or her parents private lives. Like Mukerji, before me, I too left Calcutta feeling obliged to respect their desire for privacy.

Conclusions

I have narrated my findings. I leave it to the reader to judge various issues on their merit. As far as I am concerned. Mukerji did reach great heights. His achievements in the biology of reproduction dwarf all other achievements of India in this field. Unfortunately, ignorance of the medical fraternity, bureaucratic arrogance and vindictiveness, led to the loss of one of our distinguished scientists. We also lost our claim to having been the first in the world to have succeeded in not only fertilizing human eggs *in vitro* but also having successfully cryopreserved the embryo, thawing it and leading to the birth of a healthy normal baby following the embryos replacement into the mothers womb. This situation is all too familiar to many of those who have tried to climb heights where only eagles dare to reach while lesser mortals exhibit the typical Indian Crab Syndrome which results in none of the crabs kept in a basket ever reaching the top because the remaining will pull it down.

I sincerely hope that the publication of this article will at least lead to some soul searching and result in posthumously crediting Mukerji for his brilliant achievements under very trying conditions.

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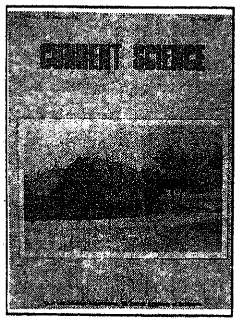

COVER. A hut constructed in a traditional way by the Nicobarese of Trinkat island. See page 541.

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In this issue

Biodiversity of Andaman and Nicobar islands: Going, going, ...

The sordid episodes of developmental activities causing enormous and irreparable damage to natural ecosystems have been described so often in the popular press, that one more tale of woe can hardly be news. Nonetheless, the grim state of affairs of Andaman and Nicobar islands documented by P. V. Sreekumar (**page 541**) deserves special attention. The many unique and remarkable features of the lifescape of these islands make them one of our most precious and most fragile natural heritages. Highlighting the immense potential of the endemic plant and animal species for enriching our genetic resources, Sreekumar rightly voices apprehensions about their imminent extinction. The most poignant of these statistics refers to the populations of the three primitive tribes – Onges, Great Andamanese and Shompens; these are represented by less than a few dozen surviving individuals. In all probability, not even a single individual will be left to participate in the centenary celebrations of Indian independence (unless the Good Shepherd Ian Wilmut decides to add them to his flock!). See this article, if only to know how much we stand to lose by neglecting the conservation of the Andaman and Nicobar islands.

N. V. Joshi

Women in science

Are women encouraged to take up careers in science? Despite increasing enrolment of women in postgraduate degree programmes and for Ph D degrees in various institutions, there are relatively few professional women scientists at the major universities and research laboratories across the country. At the higher levels of academia and science management, the balance is tilted decisively in favour of men, although the Department of Biotechnology now has its first woman secretary. There have been many attempts to increase the participation of women in scientific activities which have met with mixed success. Archana Gupta (**page 549**) analyses the experience of the CSIR extramural programme in funding women scientists. The statistics are hardly impressive, although it must be conceded that this is only a limited analysis. Funding agencies and research organizations need to come to grips with the many difficulties which women scientists face. The unrealistically low age limits for even temporary research positions, often bar women who have taken a break for purposes of raising a family, from returning to pick up the threads of a scientific career. This, coupled with poor working conditions in most laboratories can act as a powerful deterrent. There is little doubt that new initiatives are necessary such that India can benefit from the vast reservoir of talent present in half its population.

P. Balaram

Anisotropy across biological membranes

Membrane proteins have the onerous task of detecting changes in the environment of the cell and generating a first response to such changes as well as supervising all transport across membranes. In the latter role, they also serve to transmute energy into biologically useful forms. Unfortunately, these proteins have proven notoriously difficult to crystallize and there is a paucity of structural information about them. Genes for several membrane proteins have been cloned over the past decade and the genome sequencing projects underway promise a deluge of such information. Correlating sequence information with the structural and functional features of these molecules is a formidable challenge. A major contribution in this area was made by von Heijne in 1986 with his observation that the intracellular loops of membrane proteins carried more positive charges than the extra-cellular loops. This observation has since been extended to several systems. With many more sequences now available, V. Sitaramam (**page 562**) has now carried out a very extensive analysis looking for asymmetry in several parameters including charge and the tendency to form specific secondary structures. His results essentially corroborate the 'positive inside' rule with exceptions that could have functional relevance. Significantly, histidine residues, whose protonation state is sensitive to pH in the physiological range, appear to be localized close to the membrane–water interface and distributed in a manner opposite to that of the positive charges. Compilations and analyses such as these will lead to hypotheses regarding the functional role of histidines at the surface, including one proposed here. One can thus expect a spate of experiments leading from this report along with more sophisticated algorithms for the prediction of membrane protein structure.

M. K. Mathew

Current Science

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CURRENT SCIENCE

Volume 72 Number 8 25 April 1997

CORRESPONDENCE

Erosion of funding

Some of us have been watching with consternation the erosion of public funding for the IITs and IISc. It appears that these premier institutions have been singled out for some sort of experiment in financial management without any public debate within these institutions or within the general scientific community. It is not even clear if there has been a discussion in the parliament.

Not all details of the experiment are clear but what has caused surprise to most scientists is a decision to charge a fairly high tuition fee – to the tune of Rs 500 per month – from all Ph D students. Of course all institutions in the country have been advised by the Government to augment their income from non-Government sources to reduce the subsidy on higher education. In this context one can make a case for charging undergraduates in the IITs or the IIMs. These courses are stepping stones to richly paying jobs in India or abroad. That is not the case for doctoral students in science and engineering. Science at all levels and engineering at the doctoral level are increasingly unattractive options for the bright students in India. A huge tuition fee can only be a further deterrent.

It is also not clear why these premier institutes have been singled out for this experiment and why there has not been a public debate over such an important and potentially damaging step.

I have enjoyed reading in your pages lively debates on science policy. Surely this merits both an investigative reporting by *Current Science* and a public discussion by scientists, directors of these institutes, and appropriate representatives of the Government. To watch helplessly cannot be right.

J. K. GHOSH

Indian Statistical Institute,
Calcutta 700 035, India

Upper temperature limit of fungi

In their beautifully-illustrated article (*Curr. Sci.*, 1997, **72**, 306–315), H. Y. Mohan Ram and Promila Gupta write, 'Unlike certain archaea . . . and fungi which have the unusual propensity to survive and function normally under temperatures in the range of 80–100°C, most flowering plants are unable to stand temperatures above 50°C'. Whereas the stated high temperature of growth is true for the archaea, I am unaware of any species of fungi which can grow at temperature above 60–62°C. We and others (Tansey, M. R. and Brock, T. D., *Proc. Natl. Acad. Sci. USA*, 1972, **69**, 2426–2428) have searched for thermophilic fungi that are capable of growth above 60–62°C but have found no such species.

RAMESH MAHESHWARI

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Bangalore 560 012, India

H. Y. Mohan Ram and Promila Gupta reply:

We wish to admit that the temperature limit mentioned by us is valid for archaea and not for fungi. We have confirmed that the upper limit for fungi is 60–62°C by going through literature and by consulting experts. The error is regretted.

H. Y. MOHAN RAM
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Technical education – Vision 2010*

K. L. Chopra

Preamble

From time immemorial, education stands broadly for development – development of mind so that it can liberate itself from ignorance of what we are, what nature is and how we interact with nature. Very appropriately, a Vedic quotation describes the goal of education as *Sa vidya ya vimuktaye* – liberation through education. The goal of education has not changed but the gravity and multidimensionality of the goal is becoming starkly clear to all thinking human beings. With increasing demands of civilized societies whose membership continues to grow unabated, the goal of education has become associated with a goal of achieving 'good living' for the individual as also the society which pays for the education. And, thus, education gradually has assumed a purpose in the form of a social contract for the educated to serve the society.

Education for manpower development

The ability to solve the great challenges to the future depends crucially on a society which is skilled and scientifically literate. People all over the world want change. Revival for survival has spurred everybody. To meet the essential requirement of the vast population today, we need to make full and judicious use of the technological advances that have taken place. It is no more a question only of dealing with a rapidly advancing technology. It is also and even more important a question of our involvement in advancing technology rapidly enough to meet the challenge fulfilling societal needs. The role of technical education in manpower development for such a new world is thus self-evident.

Education is the most vital resource for economic development. Human resource development has a multiplier effect on utilization of all other resources. It is said that it is not the poverty of natural resources but rather the poverty of human resources which is responsible for the sad state of affairs of underdeveloped countries.

Education – technology synergism

The industrial revolution followed by a technological revolution all over the world have changed our total concept of education. Born out of necessity is the concept of technical education which goes beyond liberation of the mind of the seeker of this knowledge or the society to which the seeker belongs and to which he/she has a social contract. The new horizons are national prosperity through application of technical knowledge. This vision is best summarized in perhaps the most comprehensive and stimulating document (*The 1958 Scientific Policy Resolution of India*) ever published officially by any country in the world. It states: 'The key to national property, apart from the spirit of the people, lies, in the modern age, in the effective combination of three factors, technology, raw material and capital, of which the first is perhaps the most important... But, technology can grow out of the study of science and its applications.' This major industry paradigm shift has been dictated by the realization that science and technology have transformed numerous societies and nations into economically prosperous ones during the 19th and 20th centuries.

To appreciate the concern of our planners spearheaded by our first prime minister, Jawaharlal Nehru, the objectives of the 1958 Scientific Policy Resolution are listed below:

(i) To foster, promote and sustain, by appropriate means, the cultivation of science, and scientific research in all its aspects, pure or applied and education;
(ii) To ensure an adequate supply within the country, of research scientists of the highest quality, and to recognize their work as an important component of the strength of the nation;
(iii) To encourage, and initiate, with all possible speed programmes for the training of scientific and technical personnel on a scale adequate to fulfil the country's needs in science and education, agriculture and industry and defense;
(iv) To ensure that the creative talent of men and women is encouraged and finds full scope in scientific activity;
(v) To encourage individual initiative for the acquisition and dissemination of knowledge and for the discovery of new knowledge, in an atmosphere of academic freedom, and
(vi) In general, to secure for the people of the country all the benefits that can accrue from the acquisition and application of scientific knowledge.

The decades of eighties and nineties have witnessed phenomenal developments in the global social and political scenario, natural science, engineering and technology and industrial production. New frontiers of science have given birth to new technological revolutions such as silicon chip, computer, communication, information/knowledge, molecular electronics and biotechnology. Major world economies are increasingly being driven by 'knowledge industries'. Globalization of economies, technologies, and prevalence of competitive market forces are dominating the financial health of the new world. This has led to a new world trade organization (WTO) regulated by a General Agreement on Trade and Tariff (GATT). If these agreements become accepted world trade order, it is the economics rather than political philosophies and military powers which will rule the world. The world economies, in turn, will be determined by the generation and utilization of scientific knowledge for industrial production. Consequently, the synergetic relationship between technical education and the development of science and technology is undergoing dramatic and dynamic changes all over the world. Of course, planners in our country are quick to respond in the form of yet another document – the *Technology Policy Statement of 1983*.

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*Based on the Foundation Day Lecture delivered by the author at the Indian School of Mines, Dhanbad on 9 December 1996.

It is clear from the two documents quoted above that we are capable of defining a serious problem in the best of sermons of biblical value. Indeed, we have gone even beyond by setting up a variety of committees and commissions to examine our education policies. Such commissions as those led by our distinguished educationists such as Radhakrishan, Kothari, Thacker and Nayudamma have produced great documents of historical importance. *The New Education Policy of 1986* is the most recent voluminous document. And, the Rama Rao Committee is presently searching for new material for a new document. Has technical education or technology development benefitted from these expensive and time-consuming exercises? Why do we continue to face large-scale poverty, unemployment, illiteracy, malnutrition, disease, and a struggling economy? Regrettably, our education in general, and technical education in particular, continue to lose relevance to the needs of our society and nation. Even the best of our technical institutions in the country are barely able to keep above the level of mediocrity. We must remember that only excellence can breed excellence. And to achieve any semblance of excellence in our technical institutions, we must initiate major structural, management, financial and academic reforms.

We need to have a vision for the next century. And we must have a national commitment, industry involvement and political will to overhaul drastically our technical education system. What reforms should we contemplate? Here are some suggestions:

Proposed reforms

1. Antiquated, passive teaching must give way to active participation of students who should be respected as adults. Thus, an old-fashioned conservative attitude of parent–child relationship should be replaced with adult–adult interactive relationship.
2. We must incorporate flexibility in our curriculum through modular and bridging courses. Taking appropriate modules together to earn a degree should be as much a responsibility of the taught as that of the teacher. Students must be allowed to exercise their judgment on what they want to learn and at what pace. As long as students pay for their education, restriction on their pace and the period required for completing a degree must be minimized as far as possible. In some ways and in some cases a 'cafeteria' style for 'pick and choose' would be desirable. The concept of a broad-based engineering degree (e.g. basic engineering) without any particular specialization should be experimented within selected institutions for selected candidates.
3. If technical education is to achieve employability, redeployment, skill acquisition, and attitudinal changes, multidisciplinary and interdisciplinary courses cutting across departments/centres must be encouraged.
4. Four-year duration for a B Tech/B E degree and 1.5 years for M Tech is not adequate for providing education of globally benchmarked standards. Flexibility through modular courses should take care of the problem. Work experience between semesters with flexibility of taking breaks should be an accepted policy. Facilities for part-time registration of students should be provided.
5. Both the undergraduate and postgraduate education must insist on a substantial experimental or technological project based on realistic if not real-live industrial problems.
6. Conventional laboratory experiments be replaced, at least partially, with challenging group projects aimed at fabricating devices/instruments/products. National laboratories (e.g. CSIR, DRDO, ICAR) and industrial R&D institutions should be given due status, recognition and incentives for getting involved in the laboratory training programmes. Such programmes must carry a lot of weight/credit towards a degree.
7. Integrated M Tech/M S degree courses, particularly in multidisciplinary areas like materials, energy, instrumentation, building technology, biotechnology, engineering physics, industrial chemistry, geotechnology, cryogenics and food engineering should be introduced. As an incentive for bright students to undertake higher studies, the dual-degree concept allowing two B Tech or one B Tech and one M Tech degrees in the same or related areas should be allowed.
8. All students of technical education must be exposed to such new courses as life science and life science-based technologies, reliability and quality and intellectual property rights, ethics, technology management entrepreneurship, etc.
9. Science based know-why engineering education must be supplemented with know-how technical aspects. Design and manufacturing courses must receive due recognition.
10. New pedagogical tools should be employed to impart multifunctional skills, quality consciousness, team and group spirit, healthy attitude towards innovation, etc. Our education must inculcate 'technology patriotism' in our students.
11. Institutions must have simple internal mechanisms to drop some courses and adopt new ones. Excellence demands rapidly changing and responsive system of education. A good institution should innovate unconventional courses and popularize them among students. A suggested list of such courses is: Agro-based technologies (aquaculture, horticulture floriculture, post-harvest processing), technology assessment and forecasting, biotechnology, energy and environment management, green technologies, natural products, nanotechnologies, composite materials, value-added minerals, patent laws, total quality management, human resource management, project management, culture and tourism and entertainment industries, etc.
12. Excellence cannot be achieved without highly motivated trained, active and professionally compe-

tent teachers. Introduction of M Tech and Ph D courses for Technical Teacher Training programmes must be introduced in selected institutions.

13. All teachers in technical education must be required to be involved 50% of the time in industrially oriented R&D, consultancy, entrepreneurship, or book writing projects. Industrial exposure of at least one year must be made mandatory for teachers.
14. A dynamic faculty in a technical institution must be allowed to serve private/public industries in technical or managerial capacities concurrently with teaching on time-sharing basis. Both students and faculty should be allowed to earn consultancy fees without any upper financial limits as long as the professional assignments do not suffer. Establishment of Science & Technology Entrepreneurship Parks and setting up of enterprises by the faculty as role models should be allowed by educational institutions.
15. Free or highly subsidized education is not conducive to generating excellence through curiosity, initiative, innovativeness and competitiveness. Students must be given flexible opportunities to earn while they learn and learn while they earn. In the matter of consultancy and IPR, students should be recognized as legal partners.
16. Through the availability of information highways, on-line distance education, and virtual campus (of national institutes), cross registration for courses by interested students and recognition and transfer of credits earned through such electronic media should be an accepted mode of technical education.
17. That we have the third/fourth largest technical manpower in the world is a misleading statement. Actually, our number of technical persons relative to our population is somewhere 25 to 50 times smaller than that obtained in various developed countries. Educating larger number of students in a population edging towards a billion mark calls for high-tech solutions through information technology which is changing the mode of delivery of technical education dramatically. The open university and distance learning systems using communication satellites, wired and networked computers and multimedia facilities, cyber space and cellular telephones provide a new and powerful interactive medium for education of masses. A state-of-the-art national educational information network (such as ERNET) with large bandwidth and several Mbps speed based on ATM switches at a discounted tariff are absolutely essential to provide on-line distance education, and shared electronic libraries/databases on a country-wide basis.
18. The need for updating the knowledge and retraining of technical manpower from time to time to cope with the rapid development in technology is urgently felt. Therefore, technical educational institutions must devise continuing education packages for specific requirements. Indeed, this informal and non-formal mode of education must receive immediate attention and recognition through a formal certificate/diploma/degree.
19. Excellence in technical education must be certified by peers and autonomous agencies of high credibility. Accreditation by any agency (such as UGC and AICTE) established by any Government cannot be taken seriously. Our National Academies and Industrial Organizations are well equipped to do credible job on payment basis. We must have periodically generated ratings for individual departments as also institutions. Such accreditation must have global benchmarking so that we can compete in the very lucrative international 'education industry'. Knowledge is a saleable and marketable commodity to-day. Education is a very respectable business. Countries such as USA and Australia earn billions of dollars by selling education worldwide. Our technical education must be good enough to attract higher calibre foreign students from advanced countries. We must internationalize technical education not for money alone but more importantly for international benchmarking and accreditation.
20. Technical education needs to be managed professionally in a business-like manner. Both Central and State Governments must be persuaded to leave the governance of the technical institutions to the professionals. Their major role should be to facilitate than to govern such institutions. The best government is the one which governs the least. Clearly, a new governing structure having eminent persons who are actually involved in education and employment of the technical manpower is needed. Employers must have a direct stake in running such institutions.

Concluding remarks

What we do not need is further new committees or commissions to examine the status of technical education in India. We hardly need to be told what is wrong with it or what is required to gear up to become globally competitive. What we need are actions so that we can participate in the sweeping knowledge revolution. My vision sees an integrated system approach towards manpower development. I see manpower development through education which will relate itself to the economic development of the country and as an instrument of transformation for participating in the knowledge revolution.

The future belongs to us with an urgency seldom felt before – times are afire with the urge to blaze new trails towards putting knowledge into fruitful motion. With the economy geared to new challenges and opportunities, the nation is poised to take giant strides towards globalizing our economy and bringing prosperity to the society. It is 'now or never' for educationists, scientists, engineers and technologies to help achieve an industrial resurgence with a patriotic zeal for the India of the year 2010 AD and beyond. The primary key to progress lies in our technical education with excellence and relevance which will help us not only to tackle our basic problems but also earn India an honourable place in the company of front-ranking nations of the world. Let us set ourselves time-bound targets and work together confidently towards fulfilling them. Let us rededicate ourselves to the pursuit and attainment of excellence in technical education inspired by a sense of mission.

Let me conclude by a very appropriate quotation from a verse from *Gitanjali* of Rabindranath Tagore:

> Where the mind is without fear and the head is held high;
> Where knowledge is free;
> Where the world has not been broken up into fragments by narrow domestic walls;
> Where words come out from the depth of truth;
> Where tireless striving stretches its arms towards perfection;
> Where the clear stream or reason has not lost its way into the dreary desert sand of dead habit;
> Where the mind is led forward by thee into ever-widening thought and action-
> Into that heaven of freedom, my Father, let my country awake.

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Are we losing our heritage?

P. V. Sreekumar

Fragile ecosystems, aborigines and their traditional knowledge of the flora and fauna are apparently on the verge of extinction. The unique heritage on the lifescape of these pristine islands needs to be conserved.

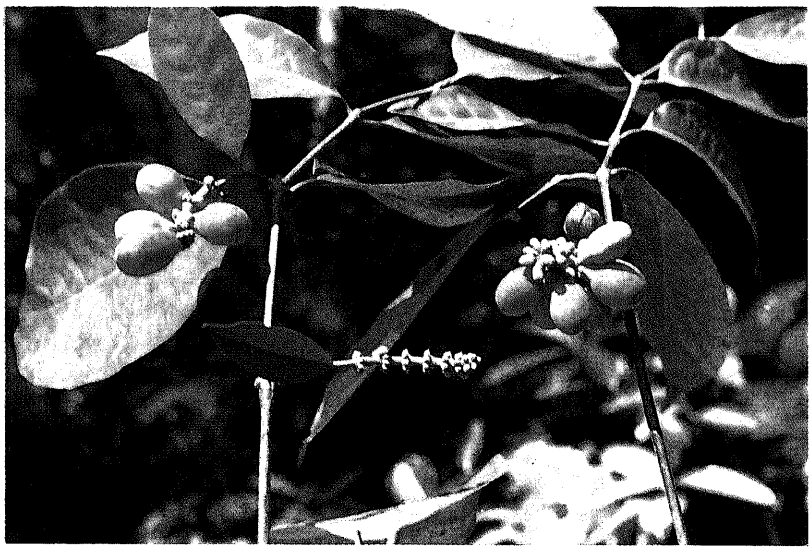
Since the age of Darwin and Vavilov, the oceanic islands have been popular for their rich and diverse biological heritage. These strips of land masses isolated by vast expanses of blue waters quite distant from any biotic interference, proximal to adaptive radiation and optimum downpour were found congenial for organic diversity, endemism, evolution and speciation, they thus had biogeographical significance. Andaman and Nicobar archipelago with over 572 pristine islands, islets and coral reefs tells a similar story in a contrasting evergreen colour over the 'then turquoise blue' Bay of Bengal. Are these ideal heritage sites now intact, or are they under threat of extinction?

Stretching to a land mass of about 8293 sq. km, with a green cover of over 86% of it, and a coast line of 1962 km, this chain of islands runs almost parallel to Malay peninsula and shows marked affinities to that as well as to Burma, Malaya and Indonesia in its analogous elements of biodiversity. These tiny islands, possessing a warm-humid climate with an average rainfall of over 3800 mm, apparently look like rugged terrain, consisting mostly of undulating hillocks just emerged or lifted out of deep sea, the highest point being the Saddle peak of the North Andaman with an altitude of only 732 m. Saldanha[1] speaks of the hypothesis behind these emergent peaks being submerged mountains related to the Arakan Yoma Range of Burma. The fragile ecosystems of these islands could be categorized into the forest, the marine and the mangrove.

Despite many exhaustive surveys to explore the rich biological resources of these islands, a comprehensive knowledge of their traditional wealth and genetic resources is ironically still lacking or yet to be catalogued. Out of the probable 2500 species of flowering plants occurring here, about 11% are endemic to this archipelago and another 11% are economically important or otherwise useful to mankind[2]. A recent census lists over 150 species of wild relatives of crop plants. Over 100 ornamental plants grow wild which could be domesticated and interpreted for further crop improvement as well as for aesthetic beautification programmes. On these tiny islands are found over 250 medicinal plants, 110 species of orchids[3] and over 75 species of wild edible fruits. Moreover these islands have over 110 species of grasses, 80 kinds of underutilized vegetables, 50 species of

Nicobarese of Trinkat island construct their huts in a traditional way – Are we not losing this heritage now?

Onges of Little Andaman dig out tubers of *Tacca leontopetaloides* (L.) O.K. and substitute them for potatoes.



The aborigines of Nicobar islands relieve their 'pain of hunger' by chewing the endosperm of *Gnetum gnemon* L. – a tree species.

A small population of *Bentinckia nicobarica* Becc. – an endemic and endangered palm in the Nancowry group of grasslands.

timber trees, 30 varieties of figs, 25 fibre-yielding plants, 20 species of lightwood for canoe making, 18 species of commercially exploitable canes[4], 15 plants as piscicides and many plants with miscellaneous uses. It is feared that they may vanish from their natural habitats unless the relevant authorities do not curb the over-exploitation of resources, unsustained developmental activities leading to habitat alternations, expansion of agriculture and indiscriminate felling of forest, etc. Nair[5] points out that during the last three decades, habitat loss of tropical forests in India is so rapid that about 30% of Indian plants are in the twilight zone of extinction.

The ecological status and origin of the grasslands of the Nancowry group of islands were considered a puzzle by many workers. This peculiar park-like 'grassheaths' spread under an area of 10,500 ha (covering islands like Trinket, Camorta, Katchal, Teressa, Bompoka and Nancowry) is also gradually diminishing. Unless atleast some of these grassland patches are protected from biotic interference like annual burning, overgrazing and conversion of forests into agriculture lands, plantation, these grassland ecosystems will slowly vanish.

The archipelago harbours 6 ethnic tribes, of which the Jarawas and Sentinelese are rather primitive and still hostile. The primitive tribes like Onges and Shompens are threatened with extinction, due to several known or unknown reasons. Nicobari lifestyle is a rare blend of tradition and modernity. they are well-known for their warm hospitality and instinctively protect and conserve biowealth. Nicobarese of Camorta and Trinkat islands, for instance, habitually start their day over a steaming cup of 'China grass tea'. Likewise, they often find pleasure in closing their laborious day or, at times, the occasional feasts, over a cup of the same tea.

Upon query it turned out to be nothing but fresh leaf blades of a *Cymbopogon* sp. The village chief further claimed that the same was a good refreshing tonic, gets rid of fatigue and also a powerful appetizer. The tribals often depend on nature for their day-to-day food, medicine and shelter. When they work hard in the forests patches, *Calamus andamanicus* Kurz quenches their thirst by its good amount of crystal clear sap and the fruits of the same and

Gnetum gnemon L. reduces their hunger. The Shompens and a few Nicobarese still depend on the fruits of screw pines (*Pandanus* spp.) for their staple food. The Onges dig out tubers of *Tacca leontopetaloies* (L.) O.K. and consume them as we do potatoes and Cassava. As stressed by Gadgil[6], information for monitoring population of thousands of species of human significance needs to be continually collected from traditional knowledge.

Over an intensive study on the ethnic tribes of these islands, T. S. Naidu (personal communication) of Pondicherry University was alarmed that the 3 primitive tribes, viz. Onges, Great Andamanese and Shompens numbering 104, 37 and 187 respectively, were on the brink of extinction and our knowledge on their primitive and traditional lifestyle would be completely wiped out from this world unless we take some steps to save these aborigines and their habitats from imminent extinction. Sami[7] reports that the present population of Great Andamanese is decimated by pneumonia, syphilis and measles to just 20. The dwelling sites of these rare tribals are threatened by various calamities and are on high extinction risks. Thus there is an urgent need to take steps to conserve their habitats, flora and fauna.

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RESEARCH NEWS

The Permian mass extinction – Are the killers from outer space?

A. V. Sankaran

> *The history of life is not necessarily progressive; it is certainly not predictable. The Earth's creatures have evolved through a series of contingent and fortuitous events.*
>
> – *Stephen Jay Gould*

It was decimation on an unprecedented scale – over 90% of marine species, 80% of vertebrates and more than 30% of insect genera vanished from Earth[1,2]. Land plants were not left untouched either, and vegetation was hit to such an extent that their decay brought about supremacy of fungi thriving on rotting and dead wood over the continents for a brief geological span[3]. The close of Permian and early Triassic, the P–Tr times, about 250 million years ago (Ma), witnessed this orgy of destruction of the living species that had so abundantly evolved over the preceding 300 million years since the early Palaeozoic era. Though the march of life on Earth has been punctuated by bouts of such extinctions (at least 5 major ones during end of Ordovician, late Devonian, end of Permian, end of Triassic and end of Cretaceous, see Figure 1), the end Permian annihilation was the worst in the planet's history, pushing to the background the much worked out and publicized Cretaceous–Tertiary (K–T) event, 65 million years ago, that witnessed the exit of dinosaurs and other genera, to a mere 47%. Over the past few years, scientists have been examining various types of geological records and other tell-tale evidences to identify some of the possible mass killers, both earth bound and beyond; however, most of the suspected agents do not appear to be the perpetrators of the P–Tr extermination, when evaluated against 'taxonomic, physiological, ecological and biogeographic patterns of extinction'[4]. Climatic changes, see-level fluctuations, dissolved oxygen depletion in oceanic waters leading to anoxia and suffocation, enhanced carbon dioxide build up on land and water, volcanism and its after effects and, very recently, a killer asteroid from outer space, are some of the agents advanced to explain the P–Tr extinction.

Many researchers believe that the end Permian extinction was due to a certain chain of natural events affecting the chemistry of air, land and water. Their onset was supposed to have been triggered by mantle changes during that period. This had resulted in ocean spreading, parting of land masses and drop in sea level leading to emergence of continental shelf, all of which precipitated severe inland erosion and burial of vast amounts of soil and vegetation[5]. The abrupt shifts in ^{12}C to ^{13}C ratios found in the sediments of this age, due to the greater abundance of

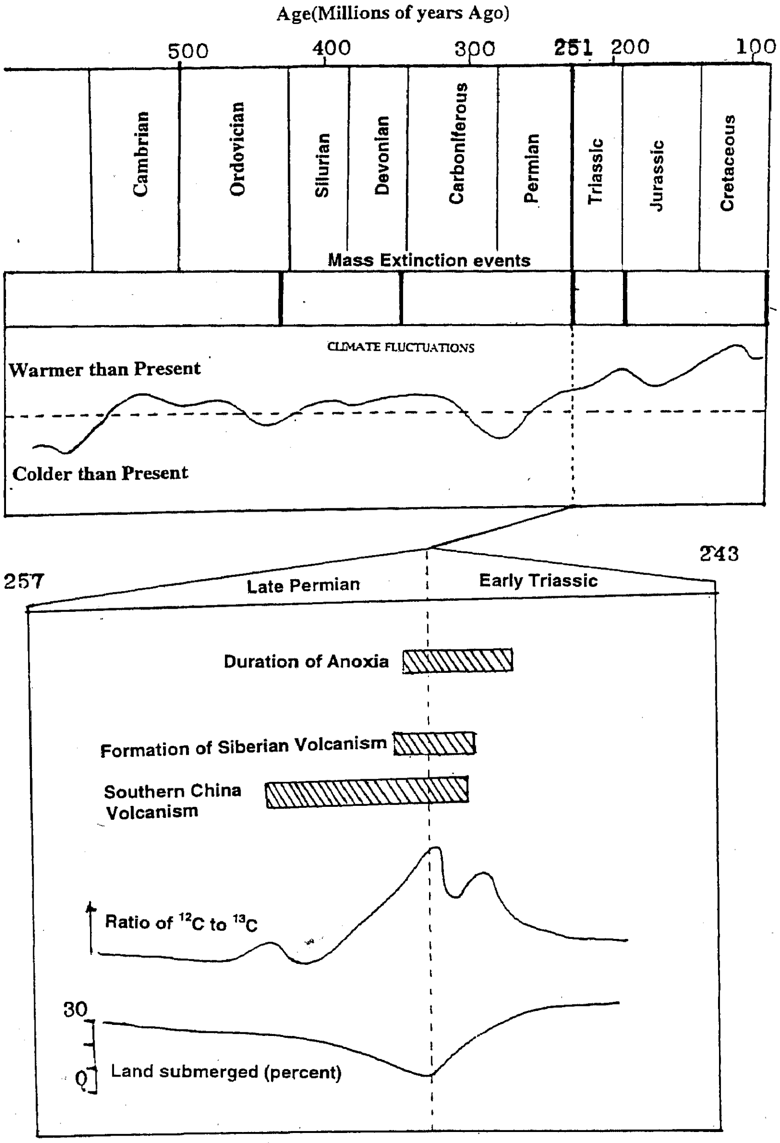

Figure 1. Environment during the Permian times and mass extinction events since the Cambrian (adapted from Erwin[1,2]).

lighter ^{12}C, are considered ample evidences for such burial[1] (biogenic carbon generally shows enrichment of the lighter isotope ^{12}C, whereas non-biogenic carbon has more of the heavier isotope ^{13}C and hence the influx of vast amounts of organic matter upsets the normal ratio in favour of the lighter isotope). Oxidation of these organic matters led to depletion of oxygen and increase of carbon dioxide in the atmosphere and consequent rise in global humidity and temperature, trends that were detrimental to the survival of life. The reduction of atmospheric oxygen had also an adverse impact on the availability of dissolved oxygen in the oceans and this gradually led to the development of anoxic (lack of oxygen) conditions and suffocation of marine life (Figure 1). Further upsets to survival of life occurred around early Triassic when marine transgression took place and ocean waters engulfed coastal habitats, killing the communities of life there.

Last year, R. K. Bambach of Virginia Polytechnique and State University, A. H. Knoll of Harvard University along with their colleagues offered a different interpretation to the chemical changes in the oceans[4]. Anoxia, they felt, cannot explain the particular patterns taken by the P–Tr extinctions and instead, the real culprit may be the increased carbon dioxide in the oceans brought about by the consumption of dissolved oxygen by the decaying organic matter. In the present times, anoxic condition does not easily develop in the ocean bottom zone due to good churning up of the oceans; the latter process effectively replaces the oxygen consumed through the circulation of oceanic currents of heavier, oxygen-rich surface waters. These currents are triggered when the waters from the cold polar regions, being denser and heavier, sink to the oxygen-deficient bottom zone of the ocean and circulate around the world before surfacing again. Such natural replenishments of oxygen (called thermohaline circulation) were non-existent during the Permian times, when the seas formed one unbroken stretch surrounding the supercontinent of Pangea. The climate, also, was too warm then to have supported an icy polar region to initiate the circulating ocean currents and bring about the churning up processes. As a result, carbon dioxide concentration in the ocean waters rose to as much as 30 times the present-day levels. Depletion of atmospheric carbon dioxide (a reverse greenhouse effect) followed, inducing a drop in global temperature, and thus initiating polar glaciation and commencement of ocean currents. This oceanic water circulation was supposed to be responsible for pushing up the relatively light anoxic bottom waters to shallower regions at the top. This influx, they contend, extinguished the marine life there, particularly those with passive respiration which were prone to accumulate carbon dioxide in their tissues, a condition known as hypercapnia or CO_2 poisoning. At this stage, exchange of carbon dioxide with the atmosphere revived, leading to global warming, melting away of polar ice and gradual decrease of thermohaline circulation once again. The adverse climatic and ocean chemistry and accompanying extinction episodes thus waxed and waned over the successive geological spans. These cyclic bouts are not much evidenced in the geologic strata of the P–Tr times but, the authors have drawn support to their hypothesis from the well-preserved geological evidences of such episodes that had occurred, at least four times, during the eras preceding the Permian period, between 800 and 540 million years ago. They feel that these on and off ocean current episodes must have continued during later periods also and brought about the decimation of life cyclically.

Advocates of volcanism cite the Siberian and south China outpourings mainly responsible for the P–Tr extinctions. The Siberian eruptions commenced during the late Permian and extended into early Triassic for about 1 million years while the south China volcanism started much earlier and was active till early Triassic (Figure 1). According to them, these prolonged eruptions altered the global climate, induced acid rains, and reduced the ozone layer protecting life from the lethal U–V radiation from the Sun. Large quantities of methane were believed to have been abruptly released which in due course got converted to carbon dioxide and water. According to one view, the large influx of methane as well as carbon dioxide may have been responsible for the mass extinction. The volcanism was also supposed to have set off extensive forest fires which destroyed the living habitats of several species, besides poisoning the atmosphere by belching out toxic effusives. Recently, Samar Abbas and Afsar Abbas of the Institute of Physics, Bhubaneswar, hypothesized that the elusive 'dark matter' pervading the universe could be the cause for the Siberian volcanism. They envisage that Earth could capture as many as 10^{18} of these particles per second whose steady accumulation and annihilation through collision with materials of Earth's interior could, over geologic time, build up sufficient heat to melt the mantle and trigger volcanic eruptions. Though these appear staggering, many scientists feel the adverse impacts of volcanism are exaggerated. Also, the commencement of Siberian eruptions is vague and some scientists even doubt whether this volcanism preceded the main mass extinction at all. Their destructive after-effects, they say, may at best be limited to a very small area only and doubt their potential to bring about the scale of mass decimation of life witnessed during the late Permian.

Though similar harbingers of death and destruction were attributed to the mass purge of dinosaurs and other species during the Cretaceous–Tertiary period, the meteorite impact view[6] had gained wide acceptance mainly due to supportive evidences gathered during the last seventeen years. These include the detection of unusual amounts of iridium in the geological formations of the times, discovery of shocked quartz at the impact crater site, and ample indications for post-impact climatic repercussions affecting the fauna and flora. None of these convincing impact-related proofs has been reported in the few catastrophic impact views[7] put out for the P–Tr extinctions.

Recently, Gregory Retallack of the University of Oregon has announced that his discovery of quartz crystals from P–Tr boundary rocks showing unusual damage to their crystallinity, points to an asteroid impact during this period[8]. The observed damage to the quartz grains appears in the form of intersecting glass-filled planar deformation fractures (PDFs) which he claims are generated due to an asteroid collision. He had collected these crystals from the P–Tr boundary near Sydney, Australia and also from the Transantartic Mountains in Vicrorialand, Antartica. These sites, though well separated today, were lying adjacent within the continent of Pangea existing at the time of the supposed impact during the Permian times. His suspicion about the asteroid connection was a sequel to the sharp changes in the carbon isotope ratios his colleagues had earlier reported from the sediments at this site. Such sharp changes are usually noticed when considerable volumes of organic material get intermixed during sedimentation, a process that can arise subsequent to an asteroid impact. The life-destroying potential of such impacts is today well known and documented by researchers who investigated the K–T event.

Though Retallack's shocked quartz grains displayed the characteristic impact induced PDFs, the typical strain effects, seen usually as colour bands under a microscope, were not reported by him. Scientists are now reexamining them for other unreported after-effects, such as the changes in the mineral's refractive index, more PDFs, intersecting at the correct angles and presence of shock-induced glass (products of rapid cooling of impact-melted rock material) at the site. Particularly sought after will be the samples showing anomalous iridium contents, typical contaminants from extraterrestrial bodies. If these findings favour Retallack's views, we have a perpetrator of the P–Tr mass extinction on the lines of the K–T event. A seismological view, yet to be confirmed, but rather supporting the asteroid hypothesis, points to the antipodal volcanism believed to be triggered by past impacts. The Siberian volcanism took place exactly on the opposite side of the proposed late Permian impact site in southeast Australia and also the massive Deccan volcanism in India erupted almost opposite to the Mexican impact site of the K–T meteorite.

Geologists have now a wide range of Permian killers, but to pin any one of them as the sole culprit, may not be acceptable as the magnitude and patterns of extinctions on land and sea appeared to be rather the outcome of a combination of adverse events taking place simultaneously or in quick succession. Even the widely accepted meteorite-related causes attributed to the K–T mass extinctions is not considered as global as made out by the proponents. It is found that the K–T meteorite's damage to life was confined to mid-latitudes only, and the impact of the collision was hardly noticeable in the then polar regions[9]. As Douglas Irwin observes 'all possibilities mentioned may have contributed. None of them alone could have caused an extinction of this size, but it was bad luck of the exquisite Permian faunas to have all these interact about the same time[1]'.

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Protective role of ashwagandha in cadmium-induced hepatotoxicity and nephrotoxicity in male mouse

Withania somnifera (ashwagandha) of family Solanaceae is a sub-tropical undershrub. It is highly esteemed as a rasayana drug in Ayurveda. Its root extract is used as tonic, alterative, aphrodisiac, narcotic, diuretic, in emaciation of children, senile debility and in rheumatism[1]. Its psychotropic, antitumour, cardiotonic and respiratory stimulating effects have also been reported[2–7]. Despite the fact that ashwagandha is an important medicinal plant, its efficacy in relation to the regulation of cadmium toxicity, if any, was not studied earlier.

Cadmium is a well-known toxicant. The toxic effects of this metal in liver and kidney tissues of mammals have already been reported[8]. Other than some chelating agents[9,10], studies on the regulation of its toxicity have been restricted to a few antioxidants such as glutathione (GSH), selenium and tocopherol[11]. We attempt here to reveal the protective effects of ashwagandha, if any, on the cadmium-induced toxicity in liver and kidney tissues of mouse.

Ashwagandha root spray dried powder was supplied by Kisalaya Pharmaceuticals Pvt Ltd, Indore, India. The dried powder was an alcoholic extract with 1.75% withanolides. The dried powder was used to prepare the aqueous extract of 40 mg/0.1 ml concentration for the treatment. 28 adult healthy colony bred Swiss male mice (approx. 3 month) weighing 30–35 g were maintained at constant temperature (27 ± 1°C) and light (14L:10D) controlled room with the provision of food (Hindustan Lever Ltd, India) and water *ad libitum*. Seven days after acclimatization, the mice were divided into four groups of seven each. Group I received 0.1 ml of normal saline per day, and served as control. Groups II, III and IV were treated with cadmium chloride ($CdCl_2$), $CdCl_2$ + ashwagandha root extract and ashwagandha extract alone, respectively. While the dose of $CdCl_2$ was 1.0 mg/day/animal[12,13], that of Ashwagandha was 1.14 g/kg body wt/day[7]. The experiment was continued for 20 days. On the last day, the animals were sacrificed, liver and kidney of each animal were removed, cleaned twice in phosphate buffered saline (pH 7.4) and immediately processed for biochemical estimations. Lipid peroxidation (LPO) was estimated by the method of Ohkawa *et al.*[14]. The liver and kidney were homogenized (10% wt/vol) in ice cold phosphate buffer (0.1 M, pH 7.4) using Potter–Elvehjem teflon homogenizer and the homogenate was centrifuged at 2000 *g* at 4°C for 30 min. The amount of malondialdehyde (MDA) formed was measured by taking the absorbance at 532 nm using Shimadzu UV-160 A spectrophotometer (Japan). LPO was expressed as the amount of MDA formed (nM) per hour per mg protein. Protein was estimated by the method of Lowry *et al.*[15]. SOD activity was determined by the method of Marklund and Marklund[16], and CAT activity by the method of Aebi[17].

Data have been finally expressed as the mean ± standard error of the mean (SEm). For statistical evaluation, analysis of variance (ANOVA) and Student's t test were used[18].

The results are summarized in Figure 1. A decrease in LPO and an increase in SOD and CAT were observed in liver homogenate when ashwagandha alone was treated. In kidney also, LPO was significantly decreased following ashwagandha treatment. On the other hand, a significant increase in the LPO in liver and kidney tissues was found

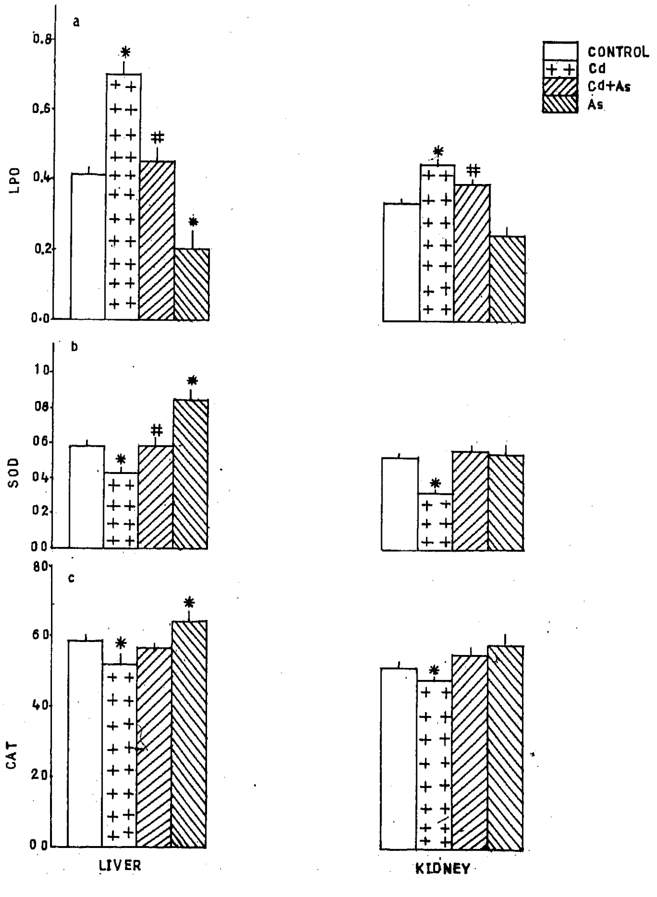

Figure 1. Effects of ashwagandha root extract (As, 1.14 g kg^{-1} day^{-1}) for 20 days on cadmium-induced hepatic and renal LPO (nM MDA formed/h/mg protein), SOD (units/mg protein) and CAT (μM of H_2O_2 decomposed/min/mg protein) activities in male mouse. Vertical lines indicate standard errors of the means. *, $P < 0.01$ compared to the respective control values. #, $P < 0.01$ compared to the values of cadmium (Cd) treated animals.

following the cadmium treatment. Both cadmium and ashwagandha-treated mice showed decrease in LPO in both the tissue homogenates when compared with that of mice treated with cadmium only. The results clearly indicate that ashwagandha is capable of reducing the toxicity caused by cadmium. Further, we found a significant decrease in SOD and CAT activities after cadmium administration and nearly normal values were obtained when ashwagandha was administered along with cadmium.

As in other studies, in the present investigation also cadmium increased tissue LPO and decreased the activities of antioxidant enzymes such as SOD and CAT which are known as endogenous antioxidative enzymes[19]. However, when ashwagandha extract was administered in the metal intoxicated mice, LPO was decreased and nearly normal values of antioxidative enzymes (SOD and CAT) were observed, indicating the ameliorating effect of this plant extract in metal toxicity.

Some antioxidants such as vitamin E, ascorbic acid and GSH decrease free radical generation and increase SOD and CAT activities. These have been found to protect the metal-induced oxidative damage[20]. However, no plant product has been reported earlier to regulate the cadmium-induced toxic effects. The present findings clearly indicate the protective role of ashwagandha extract on cadmium toxicity in mice.

It is thus suggested that ashwagandha extract may prove to be useful in the regulation of metal-induced clinical toxicity.

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Lambornella (a ciliate parasite) for biological control of *Anopheles stephensi*, the urban malaria vector

Lambornella is a hymenostome endoparasitic ciliate, two species of which namely, *L. clarki* and *L. stegomyiae*, are known to cause ciliotosis in mosquito larvae[1,2]. We reported the natural infection of immatures of *Anopheles barbirostris*, *Anopheles hyrcanus gp.* and *Anopheles philippinensis s.l.* by ciliate of genus *Lambornella* for the first time in Assam from a forest fringe village of district Dibrugarh[3]. We isolated the endoparasitic *Lambornella* from the naturally occurring infected larvae and have been culturing it in our laboratory successfully on biphasic medium of 2.8% nutrient agar with hay infusion. In our entomological laboratory the cyclical mosquito colony of the urban malaria vector, *Anopheles stephensi* (Delhi strain) is also being maintained and we report here the occurrence of epizootic of ciliate parasite belonging to *Lambornella* genus in the colony of *Anopheles stephensi* due to the accidental infection which wiped out the mosquito colony completely.

In the month of September 1996, we initially noticed the gradual mortality of IV instar larvae of *Anopheles stephensi* affecting the pupal output. The mosquito colony was being maintained in enamel pans at 27 ± 2°C temperature and 70 ± 10% relative humidity and immatures were fed on a diet of dog biscuit + yeast (60:40). Change in larval food did not stop the mortality of immatures. Meanwhile the larval mortality was also noticed in II and III instar larvae. The growth of immatures was slowed down and the larval period between two instars was lengthened to 7–8 days as compared to the normal duration of 1–2 days. The larvae became sluggish by the time of attaining the III instar stage and would not come frequently to the water surface for respiration though on tapping the pan they wriggled at the bottom. It led to the suspicion of some

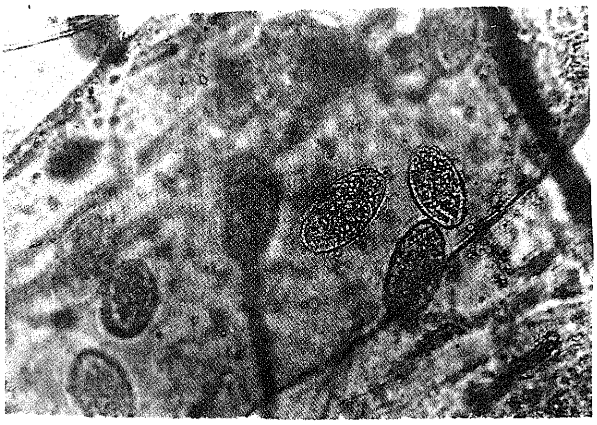

Figure 1. Infected mosquito larva with endoparasitic ciliate *in situ*. (Original magnification 1000 ×).

infection in the larvae. On examining under the microscope, mosquito immatures were found to be heavily infected with the ciliates belonging to genus *Lambornella* which were seen moving in the haemocoel (Figure 1). When examined thoroughly, the ciliate infection was noticed in all the larval instars and the extent of infection was more than 90%. Daily mortality of 10–15% larvae of all instars occurred and the total larval period from egg hatching to pupation was extended abnormally to about 25 days after which only 1–2% pupal yield could be obtained. Further, more than 95% pupae also died and finally only a few adults of *Anopheles stephensi* emerged in an nonsynchronized manner which failed to carry over the further progeny. This epizootic continued for 2–3 months and led to the collapse of the entire mosquito colony.

Our experience demonstrates that the present strain of *Lambornella* is highly pathogenic in laboratory conditions to the known urban malaria vector, i.e. *Anopheles stephensi* and that even a chance infection of it may adversely affect the colonization of *An. stephensi*. Hence it is necessary to maintain overall aseptic conditions in the insectory of this mosquito. *Lambornella* is known to form desiccation-resistant cysts to tide over the adverse conditions[1] and we suspect that the accidental infection of it in our mosquito colony passed through the cyst stages. Therefore, all laboratory ware in the mosquito insectory, particularly those used in handling of immatures, needs autoclaving to kill the cyst stages of *Lambornella*, if present. As soon as the infection of this ciliate is noticed in the immatures of mosquito colony, it is advisable to destroy immediately the entire larval population, decontaminate all laboratory ware and start fresh generation from egg stage. However, while starting afresh, the seed eggs should be from original noninfected colony because we suspect even the few adults emerging out of infected immatures may pass on the infection to the colony during egg laying as also observed elsewhere[4].

Though *Lambornella* appears to be a promising biological control agent, it will be interesting to see if it exhibits a similar degree of pathogenicity in the field conditions also. We are pursuing studies in order to work out further details to explore the true biocontrol potential of this ciliate in natural conditions and to see the various ecological limiting factors.

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Opportunities for women in science – The CSIR (extra mural research) experience

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Participation of women in technical and professional streams has shown an increase. This trend is noticed in the case of women scientists engaged in the Extra Mural Research Programmes of the Council of Scientific and Industrial Research. CSIR has encouraged women researchers to undertake R&D by sanctioning them schemes with sufficient grants.

HUMAN Resource Development (HRD) now occupies centre stage in the global arena of development. Gender equality and gender equity are emerging as major challenges. Home to over 400 million women, a country acknowledged for its unity in diversity, plurality of traditions, institutions and customs, India has always been proud of its women in every era. The 1991 census counted 407.1 million females against a male population of 439.33 million constituting just less than half (48.09%) of the total population of India (846.30 million). The sex-ratio as reported in 1991 was 927 females per thousand males[1].

India has a complex system of higher education. There are about 200 universities serving 4.3 million students. Statistics also reveal that the enrolment of women has increased almost 4 times since Independence. The enrolment of women students at the beginning of 1993–94 was 16.64 lakhs; and at the post-graduate level it was 35.1% of the total enrolment. Their performance levels have also been highly satisfactory[1].

The participation of women in technical and professional streams has also shown an increase, and there has been a noticeable growth in the number of women students in the engineering and technology streams in the IITs and polytechnics.

This trend is noticed in the participation of women scientists in the Extra Mural Research Programmes of CSIR also. The Extra Mural Research (Schemes) Programme of the Council of Scientific & Industrial Research (CSIR) provides financial assistance to promote research work in all the fields of Science & Technology, including Agriculture, Engineering and Medicine. The assistance is provided by way of grants to professors/experts in universities, IITs, post-graduate institutions, recognized R&D laboratories both in public and private sectors.

In 1990, fifty-two lady scientists and 3 emeritus scientists were awarded research schemes; in 1995 there were 55 lady scientists and 4 emeritus scientists. These scientists belong to different groups and are from all over the country. They represent life sciences, physical sciences, engineering sciences and chemical sciences.

An analysis of data pertaining to EMR schemes handled by women scientists for 1990 and 1995 reveals some interesting features. Two years' data has been taken for this as it was felt that comparison of data with a gap of 3–5 years might reflect the change that has occurred, *vis-a-vis* the EMR schemes taken by women scientists.

The emerging trends

A wide range of Institutes have had research schemes from CSIR. There are some which have been regular in sending schemes from lady researchers. Calcutta University tops the list followed closely by the Banaras Hindu University, the All India Institute of Medical Sciences and the Indian Institute of Science. That women scientists are being encouraged all over the country to participate in active research is apparent from the fact that schemes are being supported in such lesser-known institutes as Dunger College, Rajasthan, and Goa University, Goa.

Calcutta University deserves special mention. The women scientists of this university have not only been active researchers but have also shone in their sphere of research. There are 2 CSIR principal investigators who are Shanti Swarup Bhatnagar awardees and also fellows of the Indian National Science Academy. Another Shanti Swarup Bhatnagar Awardee to have a CSIR scheme is from the Indian Association for the Cultivation of Science. There are 2 FNAs from Garhwal University and the All India Institute of Medical Sciences respectively too.

Life sciences, that is medical sciences, plant and animal sciences has the largest number of schemes with

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Table 1. Trends in (first year's) research grant in schemes to women scientists in 1990 and 1995

	Rs 10,000 to 25,000				Rs 25,000 to 50,000				> Rs 50,000				No. of research fellows			
	Cont.		Eqpt.		Cont.		Eqpt.		Cont.		Eqpt		0–1		2 to 4	
Subject	1990	1995	1990	1995	1990	1995	1990	1995	1990	1995	1990	1995	1990	1995	1990	1995
Chemical sciences	6	1 (–5)	1	2 (+1)	3	9 (+6)	1	5 (+4)		2 (+2)	1	1	6	11 (+5)	4	(–4)
Physical sciences	1	(–1)		1 (+1)		5 (+5)					1	3 (+2)	1	5 (+4)		
Engineering sciences	1	(–1)									1	(–1)	1	(–1)		
Earth sciences					3	1 (–2)	1	(–1)				1 (+1)			3	1 (–2)
Mathematical sciences		1 (+1)								1 (+1)		1 (+1)		1 (+1)		1 (+1)
Medical sciences					2	(–2)		2 (+2)	1	6 (+5)	3	3	1	1	2	3 (–1)
Animal sciences	5	(–5)	1	1	14	4 (–10)	6	4 (–2)		8 (+8)	7	5 (–2)	14	9 (–5)	5	3 (–2)
Plant sciences	7	(–7)	3	3	7	12 (+5)	4	7 (–3)		5 (+5)		2 (+2)	9	11 (+2)	5	6 (–1)
Emeritus scientists	2	(–2)	1	(–1)	1	3 (+2)			1	1			3	4 (+1)		

(–) and (+) value indicate decrease and increase in trend respectively.
Base year 1990.

women as principal investigators. Chemical sciences follow close behind. There were more schemes in physical sciences and even mathematical sciences in 1995 as compared to 1990. The same holds true for emeritus scientists also.

It has been suggested that irrespective of the level of education, boys have an advantage over girls in terms of access, retention and future use of their training[2]. Moreover the education of girls continues to be influenced by a range of factors which are considerably different from those which affect boys' education. As the trend is moving from engineering subjects to life sciences with emphasis on hunger, disease and basic thirst for knowledge, women are pitching in strongly.

It has often been felt that girls are not given the same access to computers, microscopes and other essential tools as their male counterparts[3]. CSIR funding has been given adequately so that the goals can be achieved. One notices that with the increase in prices of chemicals especially biochemicals and enzymes which are necessary for life sciences, the level of funding has also increased. More researchers are getting funds between Rs 25,000 and Rs 50,000 or more for chemicals, consumables, etc. (Table 1). Chemical sciences have more contingency funding in the Rs 25,000 to Rs 50,000 category (+6) or even > Rs 50,000 category (+2) in 1995, as compared to 1990. Physical and engineering sciences also show a similar trend. But life sciences and mathematical sciences show an increase in contingency funding heading towards > Rs 50,000 (in the first year). Emeritus scientist contingent grants have now gone to the Rs 25,000 to Rs 50,000 (in first year) category. Similarly, equipment grants show that more schemes have been given equipment grants in 1995 as compared to 1990, though there are more in the Rs 25,000 to Rs 50,000 category than > Rs 50,000 (in first year) category. Since our mandate is Human Resource Development, our women researchers are also training future scientists and the number of research fellows sanctioned in schemes have also increased.

Conclusion

The notion that women should stay at home and mind the children, while men are the bread winners is an old-fashioned idea[3]. Science and technology have liberated men as well as women from many erstwhile irksome tasks. Inertia, however, is as evident in societal changes as in Newton's laws of motion, and even today society frequently acts as if it can afford to underutilize the abilities of half of its citizens. Although many formal barriers to women's participation in science have fallen, cultural attitudes and antagonisms remain. Various socio-cultural factors and lack of requisite educational and other facilities to enable them to opt for education in these fields seem to account for the low participation of women in careers in engineering, science and technology, particularly when equal opportunities are provided by the governments in various forms. Social attitudes about girls' education are also responsible for the situation; parents mainly place a higher value on the education of their sons than their daughters.

Thus despite the changing gender roles, there are women who have accomplished the Herculean task of bringing up children and building reputations as successful scientists. As society searches for solutions for global problems in need of scientific output, we cannot afford to lose the potential of women's brain power. A biological clock that requires women to make decisions about a family in the same years that their commitments to research must be pronounced, makes pursuit of an academic career difficult indeed[4]. Many things can be done, and are being done with varying degrees of success to ease difficulties faced by women as they juxtapose a career in science with the wish to raise children.

To help its women fellows/associates, a period of absence up to 3 months on grounds of maternity with full stipend is given by CSIR. A relaxation in age limit up to 5 years for applying for these posts is also given to them. Also some travel concessions have been provided. It has also been generally agreed that in case of women scientists aspiring to return to science after fulfilling their family obligations, the condition of 'publication during the last five years' would not be insisted upon.

Despite changes in attitudes, society still places on the shoulders of women the major role in home building, and that combined with their biology means that the greatest domestic stress on women occurs in conjunction with the greatest scientific stress[4]. Child-bearing and rearing years coincide with the 'assistant professor' years. A special mention must be made of the emeritus scientists who have been the pioneers of the changing scenario and have successfully sailed through all these, and are still active.

It is heartening to note that with all this, women researchers are still coming forward to ask for grants in a supposedly male-dominated society, as evident from the Table 1.

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Planning for science and technology and its relevance to national security*

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India is one of the few countries which has been observing remarkable restraint on military expenditure. In spite of that, the planners and managers of our economy brought us to the verge of bankruptcy.

AS an observer I have been deeply disturbed by the lack of understanding on this area vital to our national security and my approach to this theme is essentially as a strategic analyst. Though we have been boasting about availability of a vast reservoir of scientific manpower and we have a few achievements to our credit in agricultural production, nuclear energy, space and missile technology, yet considering the potential this country has, our achievements in science and technology lag far behind our expectations and capabilities. I was recently in South Korea and visited the Hyundai automobile factory. They started licensed production in 1967 and today they ranked tenth in the whole world in automobile technology and talk of billions of dollars of R&D investment and prototype developments from their indigenous R&D on electric cars and other advanced type of automobiles. In steel, electronics, communication equipment and shipbuilding, they started a couple of decades after India in a country totally ravaged by war and yet today they are a global player. That set me thinking on this subject of relevance of science and technology to national security. When people talk of national security in India, especially those in the economic ministries, they think solely in terms of military security. Then they give a low priority to military security and argue that the country should focus on economic development since the real security is in economic development.

Today security has a much wider connotation than military security understood in the traditional sense. A nation must have economic security, food security, energy security, environmental security and political and social security. India is one of the few countries which has been observing remarkable restraint on military expenditure. In spite of that, the planners and managers of our economy brought us to the verge of bankruptcy in 1991. After four decades of economic planning, this country is still one of the poorest in the world in terms

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*Based on the popular lecture given on November 3, 1996 at the Annual Meeting of the Indian Academy of Sciences, Jodhpur.

of per capita income, 40% of our population is still illiterate, the country still depends on foreign aid, licensed technology, heavy imports of energy source materials and lacks basic infrastructure in terms of power, transportation and communication. It is only in food production that we have managed satisfactorily. The country is worse off in terms of security with insurgencies, terrorism, inflow of small arms and narcotics. While what has been achieved – building up a state of some 500 millions above poverty level in a period of forty years – is not to be dismissed lightly, yet it is quite clear that our planners have a lot to introspect about their failures of the last four decades. In these circumstances, it is not surprising that the planning process in India has not led to this vast country with one fifth of world's population registering any global achievements and making it a global player in any major area of technology, except to a limited extent, computer software. Our performance is miserable even in an area like sports. If our Olympic team wins a single bronze medal, that is cause for great celebration. Sri Lanka which came into cricket only in the last two decades, is able to beat us hollow. Therefore our non-achievement is not something related to one particular field or the other. It spans the entire range of this country's activity. Therefore the lack of planning in the fields of science and technology should be viewed against the overall context of the country's lack of interest in achievement.

The first Prime Minister of India, Jawaharlal Nehru emphasized the need for developing science and technology and took personal interest in the scientific departments. Under his direct patronage the Council of Scientific and Industrial Research expanded and the Atomic Energy Commission was set up. The leading scientists had direct access to him. Unfortunately the Nehruvian tradition of scientific departments receiving the direct patronage of the Prime Minister later on turned out to be a disadvantage. Nehru's successors did not have his vision and were not prepared to devote as much time and interest to the advancement of science as he did. Perhaps it was not wholly their fault. The burden on the Prime Ministers has increased manifold. Logically they should have handed over the responsibility to a very senior cabinet minister who would have had adequate political weight and got things done. The Prime Minister continuing to be in charge of these departments and not being able to devote attention to them has led to their neglect. There have been occasions when the heads of scientific departments could not even meet the Prime Minister for months: The creation of the Prime Minister's Secretariat (now called the Prime Minister's office) raised one more barrier between the Prime Minister and the scientists.

The planning process in India, started with great hopes in the fifties, became a routine mechanical linear extrapolatory exercise after the third plan. Plans were put forward without any financial or political commitments. The Sarabhai plans on atomic energy or on space illustrate this kind of non-serious approach to planning on science and technology. The Planning Commission increasingly tended to get involved in implementation and there was less and less perspective planning especially in science and technology. As the planning process turned into permit license quota raj and the public sector became a milch cow for the ruling political party, the emphasis increased on import of technology. Product improvement in public sector production units was not generally encouraged. Successive imports of technology were preferred to indigenous R&D. When the country obtained licensed technology there was no attempt to establish R&D facilities associated with the technology. Neither our public sector nor our private sector thought in terms of being a global player and consequently there were no attempts at competing with global standards in quality and sophistication. There was no pressure on industry to have R&D to keep up with advances elsewhere in the globe. The protected domestic market meant there was no need for industry to exert itself. R&D did not become an engine for industrial innovation and competition. In turn the R&D had to rely exclusively on government funding. The indifference of the State to promote R&D from the seventies onwards was reflected in inadequate funding. This led to the phenomenon of brain drain when the brightest young minds started to emigrate to the US and find opportunities to exercise their talents in a congenial atmosphere there.

During this period there were also grave mistakes committed in R&D management in India which had an adverse impact on the credibility of indigenous R&D. Let me illustrate this with examples from defence with which I am more familiar. I have no doubt it applied to other fields as well. R&D projects were undertaken by understating costs and promising to reach a combination of performance characteristics totally unrealizable. This was done with the best of intentions to promote indigenous R&D. The result was initiation of R&D at inadequate levels of effort, upward revision of costs, prolongation of the R&D project much beyond the optimum period and failure to meet the promised performance characteristics. Because of long delays in R&D effort, imports were resorted to and indigenous R&D lost credibility in many instances. The major blame for this state of affairs must be laid at the door of generalist policy makers and users who are unfamiliar with R&D management and global R&D costs. But the scientific community must also share a part of the blame.

This is the overall situation in which India has decided to join the globalization process. There can be no two opinions that the globalization process had been unduly delayed. China, in spite of its Maoist past started its marketization and globalization some 12–13 years earlier and has gone about it in a massive way. The question is not whether globalization and marketization is right or wrong. It is inevitable and therefore the issue facing the country is how to carry out globalization with the least destabilizing effect on our society. In this respect this country faces a major cultural hurdle. Devising a strategy to carry out globalization in a cost-effective way needs a careful assessment about the international political, economic, and technological present and future. The Indian establishment, political, bureaucratic and scientific ones are averse to disciplined structured decision making based on systematic assessments of present and future situations. Adhocist and personalized decision making is the normal one in India. Therefore, we face problems in formulating an optimum strategy for globalization of Indian economy and technology. For the same reasons the profound changes that have come about in the international system which make R&D as one of the dominant currencies of power have not been adequately appreciated.

The Clauswitzian era in which war was considered a viable instrument of politics is now over. It is recognized that there are no worthwhile political, economic and other objectives which would justify either a nuclear war or even a high intensity conventional war, though there will continue to be threats of use of force – both with sophisticated conventional weapons as well as nuclear weapons. Force will be resorted to only in extremely asymmetric situations where a dominant country can use it against a weak country without fear of retaliation or incurring significant casualties on one's own side. There can also be terrorist use of force when the perpetrators of terrorism can inflict spectacular damage without exposing themselves. The first development leads to more and more sophisticated weapons, weapon delivery systems and surveillance systems. The second development creates a sense of insecurity among the societies hit and tends to disrupt its effective functioning. Because war is no longer viable, states which want to damage a particular neighbour tend to resort to terrorism to hurt and damage the neighbour.

Therefore, even as India tries to globalize its economy and become a major market which would lead to its becoming a major economic player in the international system, those who are not well disposed towards this country are likely to attempt to ensure that India does not project an image of stability and security which would make it an area attractive for foreign investments. In this respect security and fast economic development are symbiotically related and the old adage of choice between guns and butter popular before the international system got globalized and when wars were considered viable instruments of policy among nations is now totally inapplicable. Without optimum security and stability, there can be no orderly economic development. Development promotes security and stability but the latter are also pre-requisites for development. If a number of cities of India were to be subjected to the experience of Mumbai on 12 March, 1993 we can forget about foreign investments in the country. All the industrialized nations of the world are under the protective umbrella of a hegemonic power and so are also most of the 'tiger' economies. The latter combine high growth rates with high defence expenditure exploding yet another myth. As China's economic growth speeds up, its defence expenditure also grows rapidly.

While sophisticated military technology and nuclear weapons are still international currencies of power, their utility shows signs of reaching their limits. Unfortunately, as of today, the nuclear weapon powers attempt to project the nuclear weapons as the ultimate currency of power. In reality, the ultimate currency of power will be R&D capabilities of a nation. The Japanese boast that while the US may have conducted and won the high technology Gulf War, most of the equipment used in the war incorporated microchips made in Japan. There are ideas of using dominance over space and information technology as instruments of hegemonism over earth. Since it is expected that in the third or fourth decade of the 21st century China may catch up with the US as world's largest economy, the latter feels that the only way of asserting its pre-eminence in the global system is through its R&D. One of the papers prepared for the conference on 'The role of science and technology in promoting national security and global security' in the US by the White House Office of Science and Technology policy says: 'Since World War II, US military superiority has been based on our technological advantage, and technology will be even more important in the unpredictable security environment we now face. For technology to be an effective bulwark, however, we must abandon our reliance on separate and increasingly isolated defense industrial bases. We must recognize that commercial industry, not the military, is the driving force behind many advanced technologies today.' Unlike the US, India has no desire to be hegemonistic in military terms but promotion of R&D is essential for India in terms of its national security defined in the broadest possible terms.

India can envisage its future role in the globalized international system in two alternative ways. With its large market and a reservoir of relatively skilled labour available at low wages, India can be the base for a large number of foreign multinationals. It will grow economically, jobs will increase and it will be a major trader in the world. It will be better off compared to its present situation but will still not be a major player in the international system. Such an India living next door to a fast-growing China as a rival hegemon to the US will have to choose between the accommodation with one or the other. The other alternative is an India which not only grows economically as a market hospitable to foreign investments but applies its R&D skills and takes advantage of its market size and its competitiveness to be a major actor with its own multinationals spreading out into the rest of the world. Such an India can aspire to be an independent actor not subject to the hegemony of either China or US.

Some assessments envisage that by 2020 AD the leading seven economic powers of the world could be US, China, Japan, Germany, India, Korea and Indonesia. If that kind of status is aspired to by India, it cannot be on the basis of our permitting large-scale foreign investments in our country and not generating new products out of our own R&D. It is to be noted that out of seven countries listed only US, China and India play an independent role in the international system and the others have accepted the US hegemony. To be free of hegemony of US and China in a globalized international system is very much of a security issue. Globalization is perhaps inevitable and on the whole beneficial to India. But the ranking in the international hierarchy and the role India is to play will very much depend on how this country proposes to manage the globalization process.

India has certain inherent advantages. It has a large reservoir of manpower with potential to acquire high skills. It is an English-speaking country in a globalized system where English will be the international communication medium. It is a large market starting with a middle class of above 200 millions with tremendous expansion potential.

As mentioned earlier, a comprehensive view of national security will have to cover food security, energy security, political, social and economic security besides other factors. Our food production will have to keep up with the growing population and their increasing demands. Further there will be world-wide need for more food and that will present challenges and opportunities for our food production and exports. We are today a large importer of energy source material – particularly oil. It is obvious that the demand for energy source materials will increase exponentially with rapid industrialization of the Asian countries. This raises two problems – first, availability of oil at economic prices and secondly the environmental consequences of the large Asian countries burning fossil fuel and adding to the greenhouse gases in the atmosphere. Alternative sources of energy will need to be developed and there are vast R&D projects both at international and various national levels for this purpose. Energy conservation too is gaining increased attention all over the world.

Information technology is revolutionizing our way of life and the US writings indicate that intense exploitation of this technology is expected to keep the US as foremost military and industrial power. Biotechnology too is today a vast frontline area of research and promises undreamt of potential and changes in human society. One can add to this list and all these technologies have dual uses both in civil applications as well as in destructive ones. Therefore global competitiveness in future will mean competitiveness in R&D and efficiency in converting the results flowing from the R&D into industrial production and services. US, China, Japan, Germany and Korea are likely to treat R&D as the new currency of international power. While the US has the advantage of having been a leader now for fifty years and is likely to do its utmost to keep the lead, China will attempt to catch up with the US and overtake it, because of its larger human resource potential. In all these countries there is considerable emphasis on quality education and production of highly skilled manpower base. In addition, the US also tries to tap the skills available world-wide through its multinationals.

In the case of India, there is not even elementary awareness among our political leadership of the likely future strategic developments and their impact on our national security interpreted in the broadest possible sense. Since new sections of populations are getting successively empowered, their attention is solely focused on consolidating their power and very little attention is paid to overall national interests and security. In the longer term, such empowerment is a progressive development and helps to strengthen the nation and enrich the human resource development. In the interim period, however, the country is passing through a critical phase.

It is against this background that India has to consider long term planning for R&D to ensure that this country will play its due role consistent with its civilizational heritage and its population base. More than 25 years ago Vikram Sarabhai told me that in the latter half of 21st century the two foremost powers of the world would be China and India based on their human skills resources. I wonder whether there are people today who still cherish a similar dream. Since the political elite and bureaucracy tied to its chariot wheels are not likely to take the lead in this area, the responsibility will largely be on the scientific community and leaders of industry. If Samsung talks of a $200 billion turn-over and Hyundai dreams of reaching the top of automobile technology why should not our business aim high on similar lines. At present most of our business and scientific leaderships have not been conditioned to think strategically and relate our technological and industrial future to our national security and role in the world. While the scientific community is aware of the potential of certain areas of R&D and their roles in shaping the future and the business community is knowledgeable in assessing long term trends in international business transactions, there is at present no mechanism to develop a syncretic view of science and technology, globalized industrial and economic trends and strategic developments. This needs to be attempted and it would be appropriate if the scientific community would take the initiative in cooperation with private and public sector industry. Our state planning process, represented by the Planning Commission cannot deal with the R&D planning against this background since their exercise is a limited one and over the years has been reduced to a largely mechanical linear extrapolatory exercise. With the liberalization process, there is need for a large-scale indicative planning since bulk of funding for R&D in future will have to come from private industry. In turn captains of our industry will have to be sensitized to the imperative of investments in R&D. The State should be persuaded to increase incentives for such R&D investments. Yet another important area is to organize efficiently the expeditious transfer of R&D to production. Efforts will have to be made to involve the universities in a much larger scale than have so far been done.

REVIEW ARTICLE

Laboratory studies of density-dependent selection: Adaptations to crowding in *Drosophila melanogaster*

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Over the last decade or so, studies on replicated sets of *Drosophila* populations, subjected to life-stage-specific density-dependent natural selection in the laboratory, have greatly enhanced and refined our understanding regarding how these organisms adapt to crowded conditions. Fundamental trade-offs constraining the evolution of life-histories under crowded conditions have been uncovered, such as the negative association between efficiency of food acquisition and its subsequent utilization. A large suite of traits adaptive under crowded culture conditions has been identified, and the behavioural and physiological mechanisms underlying adaptation to crowding are becoming understood. These studies have yielded results that are sometimes at odds with well-established theoretical principles and have, thus, also led to a refinement of our theoretical understanding of how density-dependent selection can shape the life-histories of organisms.

THE theory of density-dependent selection represents one of the early attempts to bring population genetics and population ecology together by explicitly considering the impact that population density may have on evolutionary trends. Although the impact of density on various components of fitness had long been documented in many species[1–10], it was only in the 1960s that the basic ideas about the possible specific effects of density on life-history evolution were explicitly laid out[11,12]. The following decade saw the development of the formal mathematical theory of density-dependent selection which rapidly became one of the major components of the theory of evolutionary ecology[2–20]. A major theoretical advance of relevance to empirical biologists during this phase was the development of *Drosophila*-specific mathematical models that explicitly incorporated important aspects of the laboratory ecology of *Drosophila*[19,20]. The theoretical work done during this period clearly suggested that many of the predictions from the verbal theory of density-dependent selection[21–23] were far from accurate, because in its over-simplified verbal version, the theory had been used to explain all kinds of differences among widely different species. This part of the historical development of density-dependent selection theory has been extensively discussed elsewhere[24–27], and I will, therefore, not spend further time on it. What is more important from the point of view of this article is that the refinement of the theory gave impetus to rigorous and systematic empirical studies of density-dependent natural selection, and it is to these that I shall now turn.

Several empirical studies of density-dependent selection in the 1970s and early 1980s sought evidence corroborating broad predictions of the verbal theory by comparing extant species, or conspecific populations, in the wild[21,23,28,29]. The approach used was to document differences in fitness components among these populations, and ascribe them to inferred density differences in the past. In such studies it is difficult to unequivocally attribute any observed differences to density-dependent selection because the past history of the populations is often unknown and, moreover, there are many factors beyond the control of the experimenter. A few laboratory studies of density-dependent selection were also carried out with *Drosophila* species[30–32], but these suffered from serious experimental design problems[25,27,33,34].

Around this time, a series of studies on population growth rates at different densities was initiated by F. J. Ayala and co-workers, using populations of *D. melanogaster* maintained in the laboratory[35–37]. It became clear from the somewhat equivocal results of these studies that the ideal way to test the predictions of density-dependent selection theory would be to actually rear populations at high and low densities in the laboratory, under carefully controlled conditions, and then observe their growth rates across a spectrum of densities. Density-dependent growth rates were considered the principal measure of fitness in the formal theories of density-dependent selection[13–19], and these rates are very difficult to estimate from studies on populations in their natural habitat. Such a study on six populations of *D. melanogaster* maintained in the laboratory for eight generations at either low (3 *r*-selected populations) or high density (3 *K*-selected populations) showed that the *K*-selected populations had higher population growth rates and net productivity when measured at high densities. At lower densities, the *r*-selected populations were superior, although the differences were statistically significant only in the case of net productivity[38]. This finding supported the basic idea of a trade-off between adaptation to low versus high density (the central concept underlying the theory of *r*- and *K*-selection), but was at odds with results from a similar experiment done on the bacterium *Escherichia coli*. In the case of *E. coli*, the *K*-selected populations showed higher population growth rates than their *r*-selected counterparts at all densities, high or low[39]. Why this was so is not clear, although it is likely that the *K*-selection regime used in this experiment was, in fact, resulting in strong selection for higher maximal growth rates due to the alternation of periods of growth and starvation[40].

Once it had been seen that the *r*- and *K*-selected *Drosophila* populations exhibited a density-dependent trade-off in population growth rates and productivity[38], further studies on these populations, which continued to experience density-dependent selection in the laboratory were carried out. It was seen that the *K*-selected populations had greater larval viability and adult size (a correlate of greater female fecundity), when reared under crowded conditions as larvae[41]. At low densities, the *r*-selected populations had greater viability[41]. Although the differences between the *r*- and *K*-selected lines were greater when measured at high density, it was clear that the *K*-selected populations were adapting to high density and were, consequently, becoming somewhat inferior at lower densities, as compared to the *r*-selected lines. This observation gave rise to a series of studies that are still ongoing, in which the focus was to understand the specific behavioural and physiological mechanisms by which *K*-selected populations came to differ from their *r*-selected controls. These studies in the laboratory of Laurence Mueller, one of the early co-workers with Ayala[36,38,41] in the area of density-dependent selection, constitute the most comprehensive and rigorous empirical investigation of adaptation to crowding carried out in evolutionary ecology and will be the main focus of this review. Because the design and interpretation of these studies often depends critically on our knowledge of the laboratory ecology of crowded *Drosophila* cultures, I shall first review our understanding of this topic.

The laboratory ecology of crowded *Drosophila* cultures

The primary effects of crowding on the laboratory ecology of *Drosophila* can be grouped into several broad categories based on the life-stage at which crowding occurs and the specific aspect of the environment that is affected (Table 1). In general, the effects of larval crowding in *Drosophila* have been studied to a much greater extent than those of adult crowding[42]. The most obvious effect of larval crowding is, of course, increased intra-specific competition as a result of relatively rapid resource depletion[5,7,8]. The effects of this competition have an impact on both the larval and adult fitness[20]. One of the effects of food depletion, other than inducing higher larval mortality, is to reduce the size of the eclosing adults; smaller adult size tends to reduce future reproductive success in both the males[43] and females[44]. Knowledge of the biology of *Drosophila* larvae under food depletion also suggests possible adaptations that would help cope with larval crowding.

Drosophila larvae need to attain a critical minimum size in order to successfully pupate[5,8]. This, in turn, implies that there is a minimum food requirement for successful pupation, the magnitude of which would be a reflection of the intrinsic ‘efficiency’ of the larva in converting food to biomass. Individuals with a relatively lower minimum food requirement may, thus, be expected to have higher fitness under crowded conditions as they will be able to complete development on relatively smaller amounts of food; indeed, this was one of the predictions made by MacArthur and Wilson[12] for

Table 1. The range of phenotypic effects caused by crowding in laboratory cultures of *D. melanogaster*. Some of these effects are well established; others (marked by asterisks) may be seen only under specific environmental conditions or are putative, supported by only preliminary evidence

Life-stage at which crowding occurs	Primary effect (ecological consequence) of crowding	Secondary (phenotypic) effects of crowding	
		On larvae	On adults
Larvae	Food limitation due to depletion	Increased pre-adult mortality and development time	Decreased body size and, therefore, fecundity
	Build-up of nitrogenous wastes i) exposure as larvae	Increased pre-adult mortality and development time	Decreased body size, fecundity and longevity
	ii) exposure as adults	–	Decreased fecundity, increased longevity
	Medium becomes extremely soggy and moist* i) exposure as larvae*	Increased mortality of pupae on the surface of the medium	–
	ii) exposure as adults*	–	Increased mortality of adults coming to the medium surface
Adults	Food limitation	–	Reduced fecundity, longevity
	Medium becomes soggy and moist	–	Increased mortality of adults coming to the medium surface
	Crowding *per se* (increased interactions with conspecifics)*	–	Reduced fecundity*

k-selected organisms. Another possible strategy that populations experiencing crowding may evolve is to become faster feeders and more active foragers as larvae. Larval feeding rate, measured as the number of cephalopharyngeal sclerite retractions per minute[45], is a largely additive trait that responds to artificial selection in both directions[45,46], and is known to be correlated with competitive ability[45–47]. The extent of locomotory activity of larvae while foraging is known to be primarily determined by a single locus *for*, with increased locomotion (rover phenotype) being dominant[48–53]. The genetic control of larval digging activity (the depth to which larvae tend to burrow into the medium) has also been established: the trait is quantitative with substantial additive and dominance components, the latter being for increased digging[54,55]. Increased locomotion and digging while feeding, may help dispersal of larvae in three dimensions, thereby ameliorating the severity of competition in a crowded culture.

Another problem that affects both the larvae and ovipositing females in a crowded culture is the build-up over time of fairly high levels of toxic nitrogenous metabolic wastes like urea, uric acid and ammonia[56,57]. Larval biotic residues are known to inhibit both larval survivorship[58,59] and female fecundity[60]. Exposure to urea has also been shown to reduce survival and slow down the development of *Drosophila* larvae[56,57], as well as reduce subsequent adult size[27]. The presence of urea in the medium also inhibits female fecundity[61], and this effect has both short-term behavioural and longer-term cumulative (possibly physiological) components (A. Joshi, W. A. Oshiro, J. Shiotsugu and L. D. Mueller, unpubl. data). Increased larval tolerance to the deleterious effects of urea can be successfully selected for[62] and is known to be a largely dominant trait and affected strongly by both X-linked loci and maternal effects[63]. Much of the work on tolerance to nitrogenous wastes has been done using urea. However, recent work suggests that urea may not be the primary nitrogenous waste product in *Drosophila* cultures (D. J. Borash, A. G. Gibbs and L. D. Mueller, unpubl. data). It is not known whether, or to what extent, tolerance to different nitrogenous waste products is genetically correlated. Nevertheless, it is clear that tolerance to relatively high levels of nitrogenous wastes is likely to be selectively advantageous in crowded *Drosophila* cultures.

Yet another aspect of crowded *Drosophila* cultures, that may impact fitness in both pre-adult and adult phases, is the consistency of the medium. In a culture that contains a high density of larvae or adults, the food medium rapidly becomes very moist and soggy. In such cultures, individuals pupating on or close to the surface of the medium have an increased chance of being dislodged and drowned in the medium[64]. Similarly, the adults in an extremely crowded culture suffer high levels of mortality through drowning in the medium; this effect is more pronounced in females[42], presumably because they need to go to the surface of the medium to feed and oviposit whereas *Drosophila* males feed very little.

Compared to larval crowding, the effects of adult crowding in laboratory cultures of *Drosophila* are not very well known. Brief episodes (3–5 days) of crowding early in adult life, in addition to increasing mortality through drowning, also lower the subsequent fecundity and longevity of the survivors[42,65]. There is some preliminary evidence that this decrease in fecundity and longevity may be due to reduced accumulation of lipids (A. Joshi, W. Wu and L. D. Mueller, unpubl. data). Generally, in the first few days after eclosion, *Droso-*

phila females put on weight[42] and may be building up lipid reserves for egg production. Apparently, crowding during this time may reduce the ability of the females to build up these lipid reserves, although the evidence for this is still extremely preliminary. Another well-known effect of adult density in *Drosophila* is the reduction of female fecundity: fecundity declines almost hyperbolically with increasing adult density[1,5,66], although the steepness of the decline can be reduced by high food levels[20,25,27,66]. Moreover, this decline in fecundity is at least partly the consequence of increased interactions among adults and is not merely due to decreased per capita food availability at high densities[1,25].

Crowding and food levels during the adult and larval phases can also have significant effects on the population dynamics of *Drosophila* cultures. A detailed model of *Drosophila* population dynamics incorporating density-dependent viability of the larvae and the effects of larval crowding and adult crowding on female fecundity, predicted that population stability would be especially sensitive to the manner in which fecundity responds to adult density: a relatively rapid decline in fecundity with increasing density would tend to generate stability and vice versa[20]. This model has been shown to adequately account for the observed effects of larval crowding on pre-adult viability and adult size[67]. The prediction about population stability has also been verified empirically by showing that populations given low levels of food in the larval phase and excessive food in the adult phase (to reduce the sensitivity of fecundity to density) exhibit large oscillations in population size from one generation to the next[66]. Experimental validation of several key predictions of this model suggests that our understanding of the major factors affecting the ecology of crowded *Drosophila* cultures is reasonably good.

Adaptation to crowding in *D. melanogaster* under laboratory natural selection

From the foregoing discussion of the manifold phenotypic effects of crowding in *Drosophila*, it should be clear that there are many potential ways in which populations can adapt to crowding and enhance their fitness under high-density conditions. At the same time, it is unlikely that a population could simultaneously evolve all, or most, of the possible adaptations to crowding as a consequence of the ubiquity of life-history trade-offs that preclude the simultaneous maximization of many components of fitness. The evolution of adaptations to crowding in replicated sets of *D. melanogaster* populations, subjected to density-dependent natural selection in the laboratory, has been extensively studied over the past decade or so by Laurence Mueller and his co-workers, and this work has given us many insights into the trade-offs constraining density-dependent evolution in this species, as well as an appreciation of the subtle influences of rearing environment on responses to selection for increased adaptation to crowding (Table 2). Most of these studies have been carried out on two separate sets of populations: the six *r*- and *K*-populations established by Mueller and Ayala[36], referred to earlier, and, more recently, three groups of five replicate populations each, subjected to varying levels of larval and adult crowding (the UU, UC and CU pouplations described by Mueller *et al.*[68] and Joshi and Mueller[69]. UU lines are controls, *u*ncrowded both as larvae and as adults, UC lines are *u*ncrowded as larvae but *c*rowded as adults, and CU lines are *c*rowded as larvae and *u*ncrowded as adults).

Larval feeding rate versus efficiency at converting food to biomass

One behavioural adaptation that has repeatedly been observed to evolve in populations maintained at high larval density for many generations is an increased larval feeding rate[46,69,70]. Larvae from populations regularly kept at high larval densities typically take up to 20–30 bites more per minute than larvae from unselected populations maintained at low larval densities[47,69]. These larvae also have increased competitive ability against tester stocks, compared to larvae from unselected control populations[71]. More interestingly, there is evidence from both systems (*r*- and *K*-populations as well as CU and UU populations) that this increased feeding rate comes at an energetic cost: larvae from the crowded populations that feed faster than those from the control populations, actually require more food than the control larvae to successfully complete development[69,72]. This is contradictory to predictions from both the verbal[12] and mathematical[20] theories of density-dependent selection which predict that evolution in a crowded environment should favour increased efficiency of conversion of food to biomass. This finding is further corroborated by the observation that populations that have evolved higher larval feeding rates after being subjected to larval crowding for over 50 generations undergo a rapid (within 15 generations) decline in their feeding rates when they are maintained at a moderately low larval density[70]: such a decline upon relaxation of selection is indicative of a cost to higher feeding rates.

It is possible to argue that, in principle, larvae from crowded populations could still be more 'efficient' in utilizing food, despite their higher minimum food requirement for pupation, by either eclosing as heavier adults, or by completing development faster than the larvae from control populations. Both these simple explanations for the greater minimum food requirement for pupation shown by larvae from crowded populations have been ruled out by subsequent experiments on the

Table 2. The suite of adaptations seen to evolve in populations of *D. melanogaster* maintained at high densities in the laboratory. Some of these adaptations are well established; others (marked by asterisks) may be seen only under specific environmental conditions or are putative, supported by only preliminary evidence. Where the genetic control of the trait is known, it is summarized in parentheses (Q, quantitative, S, simple Mendelian, A, largely additive, D, major dominance component, X, X-linked, ME, maternal effects)

Life-stage at which crowding occurs	Primary effect (ecological consequence) of crowding	Traits seen to evolve under high-density	
		In larvae	In adults
Larvae	Food limitation due to depletion	Increases in larval feeding rate (Q, A), foraging activity (S, D), growth rate during active feeding larval phase*, and weight loss during post-feeding larval phase*	Increases in ability to store lipids and glycogen*, and in locomotor activity (S, D)*
	Build-up of nitrogenous wastes (exposure as larvae)	Increased larval tolerance to the toxic effects of urea (Q, D, X, ME)	No change in adult susceptibility to urea
	Medium becomes extremely soggy and moist (exposure as larvae)*	Increased height above medium at which larvae pupate*	–
Adults	Food limitation*	–	Reduced susceptibility to deleterious effects on fecundity and longevity induced by an episode of crowding early in adult life
	Medium becomes soggy and moist	–	Reduced mortality at medium surface, possibly through selection for negative geotaxis*

CU and UU populations[73]. In these experiments, larvae from crowded (CU) populations were seen to have faster feeding rates and higher wet weights compared to the controls (UU), especially during the third instar of larval development after the attainment of the minimum critical size for pupation[73]. However, the increased weight of late third instar larvae from the CU populations did not translate into increased adult weight; eclosing adults from both the CU and UU populations did not differ in wet weight. The development times of larvae from the CU and UU populations also did not significantly differ from one another[73]. Another possible explanation for the greater minimum food requirement for pupation of the CU populations could be that faster passage of food through the gut, as a consequence of faster feeding, results in incomplete absorption of nutrients. This possibility has been ruled out by showing that the time taken for food to pass through the gut of larvae from the CU and UU populations is not significantly different[69].

The results on the time course of larval feeding rate and weight gain in the CU and UU populations tend to support the suggestion made by Joshi and Mueller[69] that the CU larvae are less efficient at converting food to biomass because of the energetic cost of higher feeding rates and increased larval activity. The CU larvae have higher locomotory activity than their UU counterparts (they have mostly rover phenotypes)[74], and the greatest expression of the rover phenotype is late in the third instar[75]. It appears that the CU larvae lose their weight advantage, gained by faster feeding, over the UU larvae during the post-feeding phase prior to pupation, late in the third instar. Density-dependent natural selection in crowded cultures, thus, seems to favour efficiency in obtaining food at the expense of efficient conversion of the ingested food to biomass.

Larval tolerance to urea

Studies on the CU and UU populations have also shown that the CU populations have evolved greater tolerance to the toxic effects of urea. Compared to their UU counterparts, CU larvae have significantly greater survivorship and faster development on medium with 18 g/l of urea (a highly toxic concentration)[62]. Not surprisingly, the rate at which nitrogenous wastes accumulate in the food medium is much higher in CU cultures than in the uncrowded UU cultures (D. J. Borash, A. G. Gibbs and L. D. Mueller, unpubl. data). The mechanism by which the CU larvae are able to tolerate these high levels of urea is not yet known.

Pupation height

Pupation height, the height above the surface of the medium at which larvae pupate, offers a good example of how environmental factors can modulate responses to selection through genotype–environment interactions, which are, of course, ubiquitous in life-history evolution[76]. Mueller and Sweet[77] observed that the pupation height of the larvae from *K*-selected populations was greater than their *r*-selected controls. At that time it seemed reasonable to assume that the medium in the crowded *K*-selected cultures became very soggy and caused increased mortality of the individuals pupating on the surface of the medium, thereby selecting for increased pupation height. A more detailed study of mortality of individuals pupating at different heights above the medium in both the *r*- and *K*-populations, however, showed that even at low densities, selection should favour increased pupation height because individuals pupating on the food surface suffered extremely high mortality even at densities as low as 20 larvae per 8-dram vial[64]. When similar studies were repeated on the CU and UU populations, initially the CU populations evolved greater pupation heights than the UU controls[69]. After about 60 generations of selection, however, the difference in pupation height between the CU and UU populations was no longer discernible[69].

The explanation for these mutually inconsistent results may lie in the details of the maintenance regimes of the CU, UU, *r*- and *K*-populations and the conditions under which pupation height was assayed. It has been suggested that because the banana-molasses food used for the CU and UU populations was intrinsically softer and more moist than the cornmeal food used for the *r*- and *K*-populations, the UU populations were also experiencing selection for increased pupation height, albeit less strongly than the more crowded CU populations[69]. Since selection for the increased pupation height in the crowded cultures is of the stabilizing type[64], the CU populations reached a selection plateau earlier; after about 60 generations, the UU populations had also reached the selection plateau and the difference in pupation height between the two types of population was, therefore, no longer observed[69]. The *r*-populations were maintained in milk-bottles with a facial tissue inserted to control moisture. In these cultures, most larvae tended to pupate on the tissue rather than on the food or on the sides of the culture bottle. It has been postulated that the *r*-selected larvae, consequently, fortuitously escaped selection for increased pupation height[64]. The mortality of different pupation height phenotypes, however, was measured in vials without any facial tissue; thus, even at low assay densities, the larvae pupating on the surface of the medium suffered increased mortality[64].

Adaptation to adult crowding

Recent studies comparing the effects of adult crowding on the CU, UC and UU populations have provided clear evidence for the evolution of specific adaptations to adult crowding in the UC populations that are maintained at high adult densities. The behavioural and/or physiological mechanisms underlying this adaptation, however, are as yet not clear. The UC populations have been seen to suffer significantly less mortality than either the UU or CU populations, when subjected to 3–5 days of extreme crowding early in adult life[42]. The degree to which such an episode of crowding reduces subsequent longevity and female fecundity is also the least in the UC populations[42,65]. There is some preliminary evidence that the UC populations may have evolved a reduced propensity for the adults to go down to the surface of the medium during early adult life[42], drowning in the soggy medium is the major cause of mortality during adult crowding. More interestingly, it has been seen that the CU populations that are adapted to high larval density, are actually more susceptible to the deleterious effects of adult crowding than even the UU controls[42,65], raising the intriguing possibility of trade-offs between adaptations to larval versus adult crowding.

Conclusions

Particular areas of research within any scientific discipline seem to drift in and out of fashion in much the same way as specific hair styles or notions of what constitutes sartorial elegance. In the 1970s density-dependent selection occupied pride of place in evolutionary ecology. Shortly thereafter, it suffered a massive decline in popularity and acceptability, largely through the sloppy work of some researchers who tried to explain everything under the sun in terms of differences in density, and retreated into relative oblivion. Since then, however, our understanding of the density-dependent evolution of life-history traits in *Drosophila* cultures has been vastly enriched by the studies discussed here. Indeed, the main message from many of these studies would appear to be that the action of density-dependent natural selection can be much more subtle and unexpected than was earlier thought to be the case.

Today, we know a great deal about the behavioural and physiological adaptations to crowding in *Drosophila*[27,63,69,78–80]. The reality of a trade-off between efficiency of larval food acquisition and utilization is well established. There is clearly much more to be learned about the physiological underpinnings of this crucial trade-off. Studies on the metabolic rates of larvae from populations adapted to crowding, though technically daunting, should prove very fruitful. The physiological mechanisms of urea tolerance are still a mystery. Similarly, the energetic cost of such tolerance is yet to be determined; it is highly likely that the cost will be nontrival. There is tantalizing preliminary evidence that trade-offs btween adaptation to larval and adult crowding may exist[42,65]. Such trade-offs are likely to be moored in the physiological network connecting nutrient intake, assimilation and storage with primary biological attributes such as growth rates and the allocation of resources between reproduction and somatic maintenance. There is a possibility of multiple suites of strategies to cope with crowding in *Drosophila* and it may be that populations adapted to crowding are actually polymorphic for such adaptive suites of traits (D. J. Borash, A. Joshi, and L. D. Mueller, unpubl. data). I certainly hope that density-dependent selection, like most fashions, will return to the limelight, for there is much more to be studied in this most interesting phenomenon which, although presently unfashionable, is certainly not unimportant to evolutionary ecology.

I hope that this review has also served to underscore the fact that natural selection in the laboratory on replicated sets of populations constitutes a very powerful system for the elucidation of evolutionary trends and trade-offs[27,69,81–83]. No doubt, such studies do not address the question of what actually happens in nature. Nevertheless, they sharpen and refine our appreciation of what might and what might not be possible in evolution and, moreover, permit a dissection of the mutually inter-linked components of life-history evolution under specific ecological scenarios in a manner quite impossible under the relatively uncontrollable conditions of the field.

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RESEARCH ARTICLE

Anisotropy across biological membranes: Histidine charges oppose net charge anisotropy

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Considerable interest exists to find unique means to define and assess the inhomogeneity of the transmembrane terrain in terms of structural components such as the properties of the transmembrane proteins as an efficient explanation for vectorial transport, i.e. transport in specified direction. A number of properties of amino acids exhibit significant transmembrane anisotropy, i.e. directionality, when normalized per residue of the aqueous loops. Charge anisotropy in terms of fixed charges was reinvestigated and while the 'positive inwards' rule is obeyed grossly, classes of proteins were seen to show significant distinction among themselves such that receptors exhibit anisotropy 'positive inwards' while ATPases do not. Most interestingly, histidine exhibits transmembrane anisotropy opposite in sign to charge anisotropy among all classes of membrane proteins thus far examined.

DISPOSITION of proteins and amino acids across biological membranes is not symmetric. Certain aspects of this asymmetry are functionally obvious: the receptor proteins with external binding sites would have a larger bulk of the protein facing externally while transport ATPases would have the bulkier active site facing the cytoplasmic aspect. In specific instances, such an anisotropy lends clues to the function, e.g. charge anisotropy of membrane proteins[1]. Charge disposition among membrane proteins was originally described by Von Heijne as 'positive inside' rule which led to interesting possibilities on membrane biosynthesis and insertion of proteins into the membranes[2–4]. A more detailed quantitative analysis significantly amplifying this observation showed that the significant variable is positive inwards and not merely positive inside, based on the available data of confirmed and putative aqueous loops[1]. The direction was consistent with a functional role for this anisotropy such as the direction of proton movements: protons tend to move from the more positive side to the more negative side in biological membranes, which gave the physical basis of Mitchell's conjecture[5] that protons would move vectorially at least in the sense of a fixed direction. The directionality of the pump varies with whether the pump is the primary, the gradient generator, or the secondary, the gradient dissipator. Thus, the secondary pumps should have opposite anisotropy[5,6], do they?

Indeed several specific questions surfaced as a consequence of the above observations: (i) There being a large number of proteins now identified with putative predicted transmembrane topology, does the positive 'inwards' rule remain valid with a much larger sample? (ii) More interestingly, the distribution of internal and external charges were unrelated for individual proteins, which led to the interesting observation that charge anisotropy conserved larger variance among proteins; simply stated it meant that proteins would differ in their anisotropy rather than tend towards a common value, whose validity also needs to be confirmed among the larger sample of proteins; (iii) Co-transport of electrolytes and nonelectrolytes cannot be explained by transmembrane charge anisotropy alone and it remains to be examined what other properties of amino acids exhibit anisotropies across the membrane and of what magnitude. It would not be possible to guess, *a priori*, which properties would be involved in contributing to vectorial transport since one would imagine that different kinds of proximate forces would be, non-exclusively, involved in the mobility of different solutes, charged or otherwise. While this charge anisotropy would have a direct bearing on the coupled transport of charged species[7], it would by no means account for the degree of varied coupling between electrolytes and/or non-electrolytes seen at the membrane surfaces. (iv) While on a smaller sub-sample, the proton pumps did not differ from other proteins, it is basically unsatisfactory that such structural information is not thus far resolved, say, between primary and secondary pumps. The directionality seen owing to charge anisotropy was consistent with the primary pumps. The directionality of the secondary pumps remains to be accounted for.

We investigated the nature of the trans-bilayer anisotropy for membrane proteins for many amino acid properties[1,2]. We examined whether there exists any preference as to what kind of properties show a more significant anisotropy. We report here unique evidence that histidine anisotropy has a bearing opposite in sign to charge anisotropy, particularly among classes of membrane proteins which also vary in terms of charge anisotropy not in keeping with the 'positive inside' rule. These observations have exciting implications on an electrostatic regulation of transport and related activities at the membrane surface by protonation/deprotonation mechanisms of histidine at physiological pH. A comparison of the preferences with that observed in genetic code suggests the dominant role of long range interactions in proteins.

Materials and methods

A total of 157 non-conflicting and non-repeating integral membrane proteins were obtained. 136 proteins were obtained from SWISSProt Protein Sequence Database (December 1992 release) while 21 were collected from literature. The decreasing order of preference for the sequences was: human, mouse, rat, hamster, rabbit, dog, bovine, pig, yeast, bacteria. These proteins were also classified in the following six non-exclusive groups: 1. ATPases, (Atp, 16 proteins); 2. Cytochromes (Rdx, 15 proteins); 3. Transporters (Tra, 10 proteins); 4. Receptors (Rec, 116 proteins); 5. Proton pump proteins (Ppm, 24 proteins); 6. Non-proton pump proteins (Npm, 17 proteins). This study ignores the structure-based classification adopted by Von Heijne and considers the membrane proteins as a general case first; subclasses are based on their function. No effort was made to identify or distinguish experimentally determined structures as opposed to the predicted topologies for the simple reason that any pattern is particularly meaningful for prediction rather than for confirmation *post facto*.

The putative transmembrane domains were obtained from the sequence data bank and publications, primarily based on hydropathic plots as described before[1,2] and the external and internal segments were assigned consecutively. In view of the debate that exists on the use of hydropathic plots to arrive at transmembrane helices[8–10], extensive simulations using random amino acid sequences and random values for properties were both carried out but did not give any significant correlations as reported here.

Charge anisotropy

Net charge was computed by subtracting the number of negative charges (i.e. acidic amino acid residues of aspartate and glutamate) from the number of positive charges (i.e. basic amino acid residues of lysine and arginine but not histidine) for each of internal and external aqueous loops of integral membrane protein. The algebraic difference between net charge in internal and external regions per protein gives the direction of charges across the bilayer termed as charge anisotropy (CA), i.e.,

CA = Net internal charges – net external charges.

Definition of internal and external charges was based on a strict relationship to the biologically relevant topology as in the previous work[1–3]: for the plasma membrane, the external and internal aspects would be self-evident. The calcium transporting ATPase in the sarcoplasmic reticulum would have active site facing the cytosolic side, which is on the external side of the inside-out lumen of the vesicles. The matrix aspect of the mitochondrion would be inside, while the cytosolic side would be external. Submitochondrial particles would be correspondingly inside-out.

Histidine anisotropy

Assignment of net charge to histidine is complicated since it is the only amino acid residue which gets protonated or deprotonated within the physiological range of pH. It is therefore counted separately. Histidine anisotropy (HA) was calculated simply as: net internal histidine charges – net external histidine charges.

Anisotropy calculations for other amino acid properties

For computation of internal to external anisotropy, 23 different properties of amino acids were considered. The net value for external and internal domains was obtained as the mean property value normalized per residue. Anisotropy was computed per residue for all properties including charge and histidine densities, unlike in the case of charge calculations above, which considered only net charges. Anisotropy value for each property was then determined simply as the algebraic difference between the mean property per residue of internal and external regions. These mean anisotropy values for each protein and property were evaluated for 157 membrane proteins further.

Relationship to genetic code

The absence of certainty with regard to predictions of topology based on hydropathic plots remains a matter of considerable concern. Two alternatives may be considered. To be certain, the analysis could be restricted to only proteins with known topology, thereby severely limiting the sample size as well as losing the essence of the problem, viz. prediction. Alternatively, one could look for the verisimilitude: if the sequence information is generally correct, and the anisotropies reasonably accurate, the ranking of amino acid/properties in terms of their significance should quantitatively match some known ranks of importance of amino acid preference. Here one can use the genetic code to advantage since the assignment of codons to amino acids was shown to have excellent ranking conserving long-range interactions and other properties. Even if all amino acids/properties show anisotropy, it would be logical to consider that the magnitude of such anisotropy for each property would have a bearing on long term structural information of importance, even as long as term evolution *per se*. This problem statement has the additional advantage of being directly testable (using random numbers for properties as also random assignment of amino acids to each value of the property), valuable only if positive. The statement arises from the view that the properties of amino acid, alone and in combination, matter and not the head count of amino acids.

Results

Table 1 shows an updated evaluation of charge anisotropy across biological membranes for all proteins and also class-wise. The proteins are listed in the Appendix. Erythrocytes were taken as an example of a cell type. Essentially the results corroborate the positive inwards rule among all the proteins taken together, with adequate variance within them to justify certain uniqueness of the anisotropy that each protein exhibits[1]. However, the data clearly showed that this 'positive inwards' rule varied with the class of proteins and that ATPases could actually show a reversal of the anisotropy. This observation has little to do with the fact that the active site of ATPases would be the bulkiest among the aqueous loops since the receptors would also exhibit their bulkiness on the external side. Only Swissprot was considered for the database other than the references listed. While some controversy exists regarding the assignment of the topology[11], even when a 10 helix assignment for the calcium ATPase is considered, the fast twitch variant[12] had actually zero charge anisotropy, while the slow variant, SERCA2[13] has a weak inwards anisotropy of 4^+. Thus the primary conclusions of variable anisotropy remained valid for the overall pattern which did not depend on ATPases

Table 1. Net charge of amino acid residues in membrane proteins

Class of proteins	n	Internal (x)		External (y)		Anisotropy (A)		Correlation coefficients		
		Mean	SD	Mean	SD	Mean	SD	y vs x	A vs y	A vs x
All	157	3.00	12.6	–4.53	7.5	7.52	13.4	0.059	–0.473 ***	0.852***
Ppm	24	–1.83	10.4	–1.00	3.8	–0.83	9.1	–0.218	–0.510 *	0.951***
Npm	17	–7.29	14.3	–2.88	3.7	–4.41	12.9	–0.072	–0.313	0.970***
Rec	116	5.50	11.7	–5.50	8.2	11.00	14.2	0.190	–0.4619***	0.784***
Atp	16	–11.63	13.5	–1.63	3.8	–10.00	13.5	0.196	–0.085	0.960***
Rdx	15	2.13	4.5	–2.07	3.4	4.20	5.1	0.243	–0.468	0.744***
Tra	10	–1.40	12.9	–1.60	4.7	0.20	12.2	–0.574	–0.755**	0.970***
Rbc	9	–4.44	12.9	–4.11	5.3	-0.33	17.8	–0.464	–0.704*	0.956***

n, total number of proteins; SD, standard deviation; Classes of integral membrane proteins: All, Total membrane proteins; Ppm, Proton pumps; Npm, Non-proton pumps; Rec, Receptors; Atp, ATPases; Rdx, Cytochromes; Tra, Transporters; Rbc, Erythrocytes; Significance: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

Table 2. Property anisotropy of amino acid residues in 157 membrane proteins

	Internal (x)		External (y)		Anisotropy (A)	
Property	Mean	SD	Mean	SD	Mean	SD
Polarity	16.26	3.650	12.23	2.593	0.077	0.081***
Net charge density	0.04	0.035	-0.02	0.004	0.033	0.049***
Chromatographic index	9.17	1.068	9.82	0.751	-0.040	0.072***
Power to form alpha in the middle of helix	1.04	0.110	0.97	0.068	0.028	0.054***
Isoelectric point	6.22	0.602	5.93	0.158	0.036	0.077***
Propensity to form beta-sheet	0.95	0.092	1.00	0.994	-0.034	0.075***
Long range non-bonded energy	0.51	0.047	0.53	0.016	-0.040	0.089***
Entropy of formation	217	218	209	210	0.038	0.094***
Thermodynamic transfer hydrophobicity	1.20	0.169	1.27	0.127	-0.017	0.050***
Protein environment total non-bonded energy	1.71	0.142	1.75	0.026	-0.066	0.212***
Absolute entropy	45.8	46.0	44.6	44.7	0.029	0.096***
Histidine density	0.03	0.095	0.02	0.034	0.012	0.048**
Bulk hydrophobicity	12.5	1.03	12.8	0.187	-0.051	0.210**
Propensity to form alpha-helix	1.00	1.006	0.98	0.034	0.023	0.097**
Beta-sheeting tendency	0.97	0.914	1.01	0.093	-0.001	0.004**
Short and medium range non-bonded energy	1.19	0.098	1.22	0.017	-0.048	0.207**
Bend adopting power	1.06	0.138	1.10	0.109	-0.001	0.006**
Propensity to form beta-turn	1.00	0.999	1.02	1.022	-0.021	0.095**
Bulkness	14.71	1.303	14.90	0.623	-0.002	0.017^{NS}
Power to form alpha at C-terminal	1.04	0.121	1.06	0.062	-0.011	0.076^{NS}
Heat capacity	40.1	3.69	40.5	1.57	-0.004	0.038^{NS}
Power to form alpha at N-terminal	0.96	0.098	0.95	0.063	0.006	0.056^{NS}
Molecular weight	130.6	11.3	129.9	3.86	0.003	0.067^{NS}
pK'	2.09	0.171	2.11	0.035	0.000	0.005^{NS}
Refractive index	16.69	1.777	16.72	1.030	-0.001	0.045^{NS}

Each property is expressed as the mean of values of all residues normalized per residue for each region of the protein[15–17]. The mean and SD (standard deviation) refer to those computed for all proteins for these normalized values. Significance: **, $p < 0.01$; ***, $p < 0.001$. NS, Not significant, tested for non-zero mean using Student's t test[18].

alone. The receptors, with one or several transmembrane helices, carry a charge anisotropy quite opposite to that of ATPases of equally large magnitude which is not located in the binding domain either. The case of erythrocytes is instructive: since the actual charge of a membrane *in situ* is decided by the incidence of copies of each protein, e.g., band 3 protein which is a dominant protein, the positive inside rule manifests itself only when an attempt is made to mimic the copies of the protein to the extent the information is available.

Table 2 shows the properties of amino acids for which the anisotropy was computed and presented in the order of their significance for a non-zero mean, unlike as expected of a random value for a property. Such properties have been exploited to understand the basis of the origin of life and origin of amino acids and, depending on the understanding, combinations of each of these properties have been highlighted to be of significance by different workers in the past. Since the influence of these properties on a specific protein or a specific function remains elusive due to the sheer complexity of the problem, it was considered advisable to test all the readily-available properties for their relative ranking for significance. This objective screening of properties would permit recognition of gross patterns with relative ease without committing *a priori* to the nature of interactions in the functionality of the protein. The essence of anisotropy calculations here involves a comparison of the mean value of the property per residue since all properties exhibit anisotropy due to asymmetric bulkiness of aqueous segments in all the membrane proteins. Charge exhibits anisotropy as net value as well as charge density per residue. Approximately a tenth of the residues carry a charge while the net charge and number of histidines both correspond to a tenth of the total charged residues. It is important to recognize that these calculations rule out trivial objections based on the asymmetric bulkiness of the protein across the membrane since the anisotropy reported here is valid when expressed per residue as well as for the total protein as in Table 1. Table 3 shows the frequency of appearance of charged amino acids in each segment in these membrane proteins. Notably, while the basic amino acids exhibit higher preponderance in internal aqueous loops, histidine incidence was grossly the same internally and externally consistent with earlier reports[2,14]. These results highlight the specificity of the observation that histidine anisotropy counters that of charge in proteins such as ATPases beyond reasonable doubt.

Table 3. Incidence of charged amino acid residues* in external and internal regions of membrane proteins (expressed as percent of total residues in each region of all 157 proteins)

	Per cent incidence	
Amino acid	External	Internal
Aspartate	5.25	5.13
Glutamate	6.14	7.34
Lysine	4.78	7.05
Arginine	4.59	7.12
Histidine	2.50	2.30

*The results were restricted to charged amino acids only since the overall incidence was comparable to the published results[14].

Figure 1 *a–c* shows a plot of histidine display by these proteins and histidine anisotropy as a function of charge anisotropy. The histidine anisotropy was significant in itself as also in its inverse relationship to charge anisotropy. The relationship was even more clear when classes of proteins were considered (Figure 1 *d–f*). Though linear regression for the classes would be inappropriate due to the overlapping classifications, the proteins clearly exhibited an inverse relationship between histidine anisotropy and charge anisotropy and the large scatter was also consistent with an earlier surmise that the proteins maximize variance in the assignment of anisotropy, i.e. each protein occupies its own niche in terms of its anisotropy maximizing its spread[1]. The results were essentially same when net histidine occurrence was replaced with histidine density (i.e. per residue in each segment) (data not given).

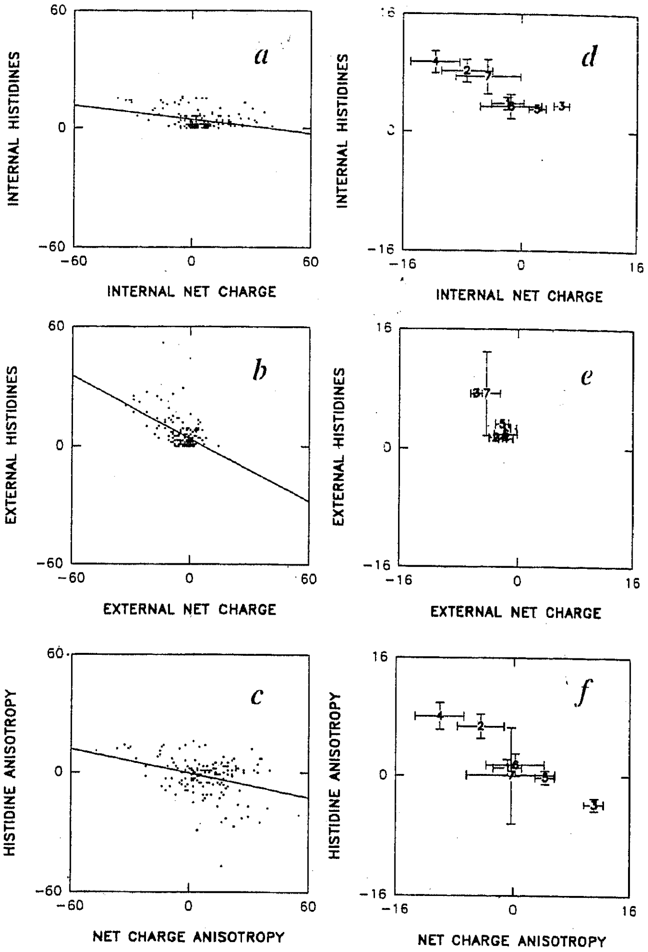

Figure 1. Relationship between histidines and net charges. *a–c*, when all membrane proteins ($n = 157$) were taken together; *d–f*, when specific classes of membrane proteins were considered. Internal cytoplasmic histidines versus internal net charges (***a***, ***d***); external cytoplasmic histidines versus external net charges (***b***, ***e***); histidine anisotropy versus net charges anisotropy (***c***, ***f***); Lines in *a–c* represent linear regressions *vis-à-vis* ***a***, $r = -0.9067$; ***b***, $r = -0.8356$ and ***c***, $r = -0.9649$ (all correlation coefficients were highly significant at $p < 0.00001$). Numbers in ***d–f*** represent classes of proteins as: 1, Ppm; 2, Npm; 3, Rec; 4, Atp; 5, Rdx; 6, Tra; and 7, Rbc; while horizontal and vertical bars represent standard error around means of each class of proteins.

Discussion

A curious observation of statistical significance with regard to charge anisotropy was that the variance among proteins was conserved among the proteins (see ref. 1). If a particular property was to serve the same purpose among different proteins, such as insertion into the membrane, it would be logical to consider that different proteins would tend to have low variance and not a high variance. This was the essence of the 'positive inside' rule of Von Heijne[2], belied by two aspects of charge anisotropy. Firstly, variance was conserved and not minimized and secondly the anisotropy exhibited definitive relationship to classes of proteins. The same arguments appear to hold good for histidine anisotropy also, which would further highlight the functionality of charge/histidine anisotropy in these proteins.

We examined whether the observed anisotropy among various properties has any order or logic intrinsic to it. One way was to test wherever such properties would be manifest, e.g. as in the genetic code. We have earlier computed the nature of assignment of codons to amino acids and ranked various properties according to the order of their conservation in the face of codon mutations[15–17]. The order of significance in anisotropy among the properties was closely related to the order of conservation observed in the genetic code($r = 0.601$; $n = 23$ properties, $P < 0.001$). Thus the observed anisotropies of properties are of substantive significance functionally and presumably also in terms of evolution. While Nakashima and Nishikawa[14] obtained a comparable incidence of amino acids and differences between the two sides of the membrane, these differences in the incidence of amino acids was not correlated to properties other than charge thus far. Anisotropy was not considered by other workers in the manner considered here. Since long-range interactions were shown to dominate in the assignment of codons to amino acids in the genetic code[15,17], comparison of the preference for the assignment of property anisotropy of membrane proteins assumes special significance. For instance, it is now textbook knowledge that the frequency of assignment of codons to amino acids has a direct relationship to the frequency of occurrence of amino acids in proteins across species. Thus long range interactions as with the genetic code playing second only to charge anisotropy in the transmembrane topology would be of functional importance in view of the similarity to the genetic code as well.

Why histidine anisotropy and why is it in a direction opposite to that of charge anisotropy? Clearly, the anisotropy of major magnitude was the net charge and not net histidine charge. This would make histidine more a probe for the electrostatic forces at the interface rather than it being the determinant of the electrostatic forces. Since small changes in the interfacial ionic strength could lead to changes in pH and vice versa, variations in histidine protonation would be a logical consequence of variations of surface pH, which in turn would offer an excellent means for coupling activity of these proteins to ionic strength. Histidine has a role as a charged amino acid as well as an aromatic amino acid while the role of histidine charge in protein function could be evaluated by pH titrimetry, the role of the amino acid itself would require a variety of experiments including titrimetry, chemical modifications and site-directed mutagenesis. If the amino acid is primarily interfacial, an attractive possibility would be the variable charge in the realm of physiological pH such that the uncharged residue could move deeper into the bilayer in the absence of the positive charge. The frequency distribution of the distance of histidines from the interface, external and internal for membrane proteins, was obtained and it could be seen that nearly 50% of all the histidines occurred at the 1st aqueous amino acid and this value reduced with the logarithm of the distance in amino acids from the interface to 50% by the 5th aqueous amino acid from the interface (data omitted, coefficient of correlation, $r = 0.94$, $n = 6$). Thus most histidines would be fairly close to the interface and could participate in interfacial pH changes. Thus the role of such histidines would probably be modulation of activity of membrane proteins as a consequence of interfacial changes of ionic strength/pH and not be determinants of such ionic changes. We are currently investigating the role of electrostatic forces at the interface and their role in membrane stability wherein variable histidine anisotropy could play a significant role. A key input into such investigations would be the remarkable pH dependence near the pK for histidine as well the influence of chemical reagents that destroy the histidine residue such as diethylpyrocarbonate. The electrostatic repulsion at the surface of rabbit sarcoplasmic reticulum (SR) vesicles at physiological pH appear to be influenced little by the carboxyl and amino groups while histidines contribute maximally; further, the contribution of histidine charges was consistent with the predicted topology of the relevant calcium ATPase of the fast and slow twitch muscle SR vesicles (unpublished results). It is hoped that analogous to various sequence specific motifs described thus far, the display of histidines at the surface of the membrane, as a pH-modulable variable, could offer a common explanation for a variety of pH dependent interactions. It is likely as well as testable that a possible role for charge anisotropy with compensating histidine anisotropy could play a role even in biosynthesis of proteins as surmised earlier. Histidine anisotropy could thus offer an additional tool for the topological prediction of membrane proteins in addition to the 'positive inside' rule, while permitting a functional perspective since the positive 'inwards' rule has emphasized the variance in the anisotropy. The relevant experimental and theoretical studies are in progress.

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Appendix

The following table lists all the proteins used in this manuscript and the subclassifications used. The predominant group of proteins are the receptors. The sub classification was overlapping depending on the possible interest in a particular class of proteins.

List of proteins used in the analysis of charge anisotropy

	Protein	Source/code	Class	Number of	
				Amino acids	Helices
1.	5-Hydroxytryptamine 1A receptor (5-HT-1A) (serotonin receptor)	5HTA_HUMAN (P08908)	Rec	421	7
2.	Activin receptor type I precursor (ACTR-I) (EC 2.7.1.-)	AVR1_HUMAN (P27037)	Rec	513	1
3.	Alpha-1A adrenergic receptor	A1AA_HUMAN (P25100)	Rec	501	7
4.	Alpha-2C adrenergic receptor (subtype C4)	A2AC_HUMAN (P18825)	Rec	461	7
5.	Apoptosis-mediating surface antigen FAS precursor (APO-1 antigen)	FASA_HUMAN (P25445)	Rec	335	1
6.	Asialoglycoprotein receptor 1 (hepatic lectin H1) (ASGPR)	LECH_HUMAN (P07306)	Rec	290	1
7.	Asialoglycoprotein receptor 2 (hepatic lectin H2) (ASGPR)	LECI_HUMAN (P07307)	Rec	311	1
8.	Atrial natriuretic peptide receptor A precursor (ANP-A) (ANPRA) (GC-A) (guanylate cyclase) (EC 4.6.1.2)	ANPA_HUMAN (P16066)	Rec	1061	1
9.	Atrial natriuretic peptide receptor B precursor (ANP-B) (ANPRB) (GC-B) (guanylate cyclase) (EC 4.6.1.2)	ANPB_HUMAN (P20594)	Rec	1047	1
10.	B-cell differentiation antigen LYB-2 (CD72)	CD72_HUMAN (P21854)	Rec	359	1
11.	B-cell receptor CD22 precursor	CD22_HUMAN (P20273)	Rec	647	1
12.	Band 3 protein of human erythrocyte; anion-exchange protein (AE1)	BAND3_HUMAN[1]	Tra/Npm/Rbc	911	14
13.	Beta-1-adrenergic receptor	B1AR_HUMAN (P08588)	Rec	477	7
14.	Beta-3-adrenergic receptor	B3AR_HUMAN (P13945)	Rec	402	7
15.	C5A anaphylatoxin chemotactic receptor	C5AR_HUMAN (P21730)	Rec	350	7
16.	Calcium-transporting ATPase plasma membrane 1B (EC 3.6.1.38) (calcium pump) (PMCA1B)	ATCQ_HUMAN (P20020)	Atp/Npm/Rbc	1220	10
17.	Calcium-transporting ATPase plasma membrane 4 (EC 3.6.1.38) (calcium pump) (PMCA4)	ATCR_HUMAN (P23634)	Atp/Npm/Rbc	1205	10
18.	Cannabinoid receptor	CANR_HUMAN (P21554)	Rec	472	7
19.	CD40L receptor precursor (B-cell surface antigen CD40) (BP50) (CDW40)	CD40_HUMAN (P25942)	Rec	277	1
20.	CD44 antigen, epithelial form precursor (CD44E) (phagocytic glycoprotein I) (PGP-1) (hutch-I) (extracellular matrix receptor-III) (ECMR-III)	CD4X_HUMAN (P22511)	Rec	493	1
21.	CD44 antigen, hematopoietic form precursor (CD44H) (phagocytic glycoprotein I) (PGP-1) (hutch-I) (extracellular matrix receptor-III)	CD4H_HUMAN (P16070)	Rec	294	1
22.	Complement receptor type 1 precursor (C3B/C4B receptor) (CD35)	CR1_HUMAN (P17927)	Rec / Rbc	2039	1
23.	D(1A) dopamine receptor	DADR_HUMAN (P21728)	Rec	446	7
24.	Dopamine receptor	D2DR_HUMAN (P14416)	Rec	443	7
25.	Dopamine receptor	D4DR_HUMAN (P21917)	Rec	387	7
26.	Dopamine receptor	D5DR_HUMAN (P21918)	Rec	477	7
27.	Endothelin-1 receptor precursor (ET-A)	ET1R_HUMAN (P25101)	Rec	427	7
28.	ERBB-2 receptor protein-tyrosine kinase precursor (EC 2.7.1.112)	ERB2_HUMAN (P04626)	Rec	1255	1
29.	EVI2 protein precursor	EVI2_HUMAN (P22794)	Rec	232	1
30.	Fibronectin receptor alpha subunit precursor (integrin alpha-F) (integrin alpha-5) (VLA-5) (CD49E)	ITA5_HUMAN (P08648)	Rec	1049	1
31.	Fibronectin receptor beta subunit precursor (integrin beta-1)	ITB1_HUMAN (P05556)	Rec	798	1
32.	FMLP-related receptor I (FMLP-R-I)	FML1_HUMAN (P25089)	Rec	353	7
33.	FMLP-related receptor II (FMLP-R-II)	FML2_HUMAN (P25090)	Rec	351	7
34.	Follicle stimulating hormone receptor precursor (FSH-R)	FSHR_HUMAN (P23945)	Rec	695	7
35.	Glucose transporter protein, erythrocyte/brain	GTR1_HUMAN (P11166)	Tra / Npm	492	12
36.	Glucose transporter, small intestine	GTR5_HUMAN (P22732)	Tra / Npm	501	12
37.	Glucose transporter-like protein, muscle	GTR3_HUMAN (P11169)	Tra / Npm	496	12
38.	Glycophorin C (PAS-2') (glycoprotein beta) (GLPC) (glycoconnectin)	GLPC_HUMAN (P04921)	Rec / Rbc	128	1
39.	Glycoprotein GP34	GP34_HUMAN (P23510)	Rec	183	1
40.	Granule membrane protein 140 precursor (GMP-140) (PADGEM) (CD62) (leukocyte-endothelial cell adhesion molecule 3) (LECAM3)	LEM3_HUMAN (P16109)	Rec	830	1
41.	Granulocyte-macrophage colony-stimulating factor receptor precursor (GM-CSF-R)	GMCR_HUMAN (P15509)	Rec	400	1

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42.	Heat-stable enterotoxin receptor precursor (GC-C) (guanylate cyclase) (EC 4.6.1.2)	HSER_HUMAN (P25092)	Rec	1073	1
43.	High affinity immunoglobulin epsilon receptor alpha-subunit (FCERI) (IGE FC receptor, alpha-subunit)	FCEA_HUMAN (P12319)	Rec	257	1
44.	High affinity immunoglobulin gamma FC receptor I 'A form' precursor (FC-gamma RI) (FCRI) (IGG FC receptor)	FCG0_HUMAN (P12314)	Rec	374	1
45.	High affinity immunoglobulin gamma FC receptor I 'B form' precursor (FC-gamma RI) (FCRI) (IGG FC receptor I)	FCG1_HUMAN (P12315)	Rec	344	1
46.	High affinity interleukin-8 receptor B (IL-8R)	IL8B_HUMAN (P25025)	Rec	355	7
47.	Histamine H2 receptor (gastric receptor I)	HH2R_HUMAN (P25021)	Rec	359	7
48.	Immunoglobulin alpha FC receptor precursor	FCAR_HUMAN (P24071)	Rec	287	1
49.	Inducible membrane protein R2	R2_HUMAN (P27701)	Rec	267	4
50.	Integrin alpha-3 (galactoprotein B3) (GAPB3) (VLA-3)	ITA3_HUMAN (P26006)	Rec	1019	1
51.	Integrin alpha-6 precursor (VLA-6) (integrin ALPHA-E)	ITA6_HUMAN (P23229)	Rec	1073	1
52.	Integrin beta-6 subunit precursor	ITB6_HUMAN (P18564)	Rec	788	1
53.	Integrin beta-7 subunit precursor	ITB7_HUMAN (P26010)	Rec	798	1
54.	Integrin beta-8 subunit precursor	ITB8_HUMAN (P26012)	Rec	769	1
55.	Intercellular adhesion molecule-1 precursor (ICAM-1) (major group rhinovirus receptor) (CD54)	ICA1_HUMAN (P05362)	Rec	532	1
56.	Interferon-alpha receptor precursor (IFN-alpha-REC)	INAR_HUMAN (P17181)	Rec	557	1
57.	Interferon-gamma receptor precursor	INGR_HUMAN (P15260)	Rec	489	1
58.	Interleukin 4 receptor precursor (IL4R)	IL4R_HUMAN (P24394)	Rec	825	1
59.	Interleukin-1 receptor, type I precursor (IL-1R1) (P80)	IL1R_HUMAN (P14778)	Rec	569	1
60.	Interleukin-1 receptor, type II precursor (IL-1R2)	IL1S_HUMAN (P27930)	Rec	398	1
61.	Interleukin-2 receptor alpha chain precursor (IL-2 receptor alpha subunit) (P55) (TAC antigen) (CD25)	IL2A_HUMAN (P01589)	Rec	272	1
62.	Interleukin-2 receptor beta chain precursor (IL-2 receptor) (P70-75) (high affinity IL-2 receptor beta subunit)	IL2B_HUMAN (P14784)	Rec	551	1
63.	Interleukin-7 receptor precursor (IL-7R)	IL7R_HUMAN (P16871)	Rec	459	1
64.	Low affinity immunoglobulin gamma FC receptor II precursor (FC-gamma RII) (FCRII) (IGG FC receptor II) (CD32) (CDW32)	FCG2_HUMAN (P12318)	Rec	311	1
65.	Muscarinic acetylcholine receptor M2	ACM2_HUMAN (P08172)	Rec	466	7
66.	Muscarinic acetylcholine receptor M3	ACM3_HUMAN (P20309)	Rec	590	7
67.	Muscarinic acetylcholine receptor M5	ACM5_HUMAN (P08912)	Rec	532	7
68.	NKG2-A and NKG2-B type II integral membrane proteins	NKGA_HUMAN (P26715)	Rec	233	1
69.	NKG2-C Type II integral membrane protein	NKGC_HUMAN (P26717)	Rec	231	1
70.	NKG2-D type II integral membrane protein	NKGD_HUMAN (P26718)	Rec	216	1
71.	Plasma-cell membrane glycoprotein PC-1 (alkaline phosphodiesterase I (EC 3.1.4.1) / nucleotide pyrophosphatase (EC 3.6.1.9))	PC1_HUMAN (P22413)	Rec	873	1
72.	Platelet membrane glycoprotein IA precursor (collagen receptor) (integrin alpha-2) (VLA-2) (CD49B)	ITA2_HUMAN (P17301)	Rec	1181	1
73.	Platelet membrane glycoprotein IIB precursor (GPIIB) (integrin alpha-IIB) (CD41)	ITAB_HUMAN (P08514)	Rec	1039	1
74.	Probable G protein-coupled receptor EDG-1	EDG1_HUMAN (P21453)	Rec	381	7
75.	Sodium/potassium transporting ATPase alfa-subunit	Pig'[9]	Atp / Npm	1013	7
76.	Sodium/potassium-transporting ATPase beta-1 chain (EC 3.6.1.37) (sodium/potassium-dependent ATPase)	ATNB_HUMAN (P05026)	Atp/Npm/Rbc	303	1
77.	Sodium/potassium-transporting ATPase beta-2 chain (EC 3.6.1.37) (sodium/potassium-dependent ATPase)	ATNC_HUMAN (P14415)	Atp/Npm/Rbc	290	1
78.	T-cell surface glycoprotein CD3 epsilon chain precursor (T-cell surface antigen T3/LEU-4 epsilon chain)	CD3E_HUMAN (P07766)	Rec	233	1
79.	T-cell surface glycoprotein CD3 zeta chain precursor (T-cell receptor T3 zeta chain)	CD3Z_HUMAN (P20963)	Rec	163	1
80.	T-cell surface glycoprotein CD5 precursor (lymphocyte glycoprotein T1/LEU-1)	CD5_HUMAN (P06127)	Rec	495	1
81.	Vitronectin receptor alpha subunit precursor (integrin alpha-V) (CD51)	ITAV_HUMAN (P06756)	Rec	1048	1
82.	4F2 cell-surface antigen heavy chain (4F2HC)	4F2_MOUSE (P10852)	Rec	526	1
83.	5-hydroxytryptamine 3 receptor precursor (5-HT-3) (serotonin-gated ion channel receptor)	5HT3_MOUSE (P23979)	Rec	487	4
84.	Activin receptor type IIB precursor (ACTR-IIB) (EC 2.7.1.-)	AVRB_MOUSE (P27040)	Rec	536	1
85.	B-cell-specific glycoprotein B29 precursor	B29_MOUSE (P15530)	Rec	228	1
86.	Basigin precursor (basic immunoglobulin superfamily) (membrane glycoprotein GP42)	BASI_MOUSE (P18572)	Rec	273	1

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87.	Cell surface adhesion glycoproteins LFA-1, CR3 and P150,95, beta-subunit precursor (CD18 antigen beta subunit) (integrin beta-2)	ITB2_MOUSE (P11835)	Rec	770	1
88.	Interleukin-5 receptor precursor (IL5R)	IL5R_MOUSE (P21183)	Rec	415	1
89.	Low affinity immunoglobulin epsilon FC receptor (lymphocyte IGE receptor) (FC-epsilon-RII)	FCE2_MOUSE (P20693)	Rec	331	1
90.	Low affinity immunoglobulin gamma FC receptor II-2 precursor (FC-gamma RII) (FCRII) (IGG FC receptor II beta-2)	FCGX_MOUSE (P08102)	Rec	283	1
91.	Low affinity immunoglobulin gamma FC receptor III precursor (FC-gamma RIII) (FCRIII) (IGG FC receptor alpha)	FCG3_MOUSE (P08508)	Rec	261	1
92.	Natural killer cell surface protein P1-40 (NKR-P1 40)	NK14_MOUSE (P27814)	Rec	220	1
93.	5-Hydroxytryptamine 1C receptor (5-HT-1C) (serotonin receptor)	5HTC_RAT (P08909)	Rec	460	7
94.	5-Hydroxytryptamine 2 receptor (5-HT-2) (serotonin receptor)	5HT2_RAT (P14842)	Rec	471	7
95.	Alpha-2A adrenergic receptor	A2AA_RAT (P22909)	Rec	450	7
96.	Alpha-2B adrenergic receptor	A2AB_RAT (P19328)	Rec	453	7
97.	B2 Bradykinin receptor	BRB2_RAT (P25023)	Rec	396	7
98.	Beta-2-adrenergic receptor	B2AR_RAT (P10608)	Rec	418	7
99.	Calcium-transporting ATPase plasma membrane (EC 3.6.1.38), brain isoform 2 (PMCA2)	ATCQ_RAT (P11506)	Atp / Npm	1198	10
100.	CD44 antigen precursor (phagocytic glycoprotein I) (PGP-1) (hutch-I) (extracellular matrix receptor-III) (ECMR-III) (GP90 lymphocyte homing)	CD44_RAT (P26051)	Rec	364	1
101.	D(1B) dopamine receptor	DBDR_RAT (P25115)	Rec	475	7
102.	Dopamine receptor	D3DR_RAT (P19020)	Rec	446	7
103.	Endothelin B receptor precursor (ET-B) (endothelin receptor non-selective type)	ETBR_RAT (P21451)	Rec	441	7
104.	Glucose transporter protein, liver	GTR2_RAT (P12336)	Tra/Npm/Rbc	522	12
105.	Growth hormone receptor precursor (GH receptor) (serum binding protein)	GHR_RAT (P16310)	Rec	638	1
106.	High affinity immunoglobulin epsilon receptor alpha-subunit (FCERI) (IGE FC receptor, alpha-subunit)	FCE1_RAT (P12371)	Rec	245	1
107.	High affinity immunoglobulin epsilon receptor beta-subunit (FCERI) (IGE FC receptor, beta-subunit)	FCEB_RAT (P13386)	Rec	243	4
108.	Integrin alpha-1 precursor (laminin and collagen receptor) (VLA-1) (CD49A)	ITA1_RAT (P18614)	Rec	1180	1
109.	Kupffer cell receptor	KUCR_RAT (P10716)	Rec	550	1
110.	Lysosome membrane protein II (LIMP II)	LIM2_RAT (P27615)	Rec	477	2
111.	Metabotropic glutamate receptor precursor (GLUGR)	GLGP_RAT (P23385)	Rec	1199	7
112.	Muscarinic acetylcholine receptor M1	ACM1_RAT (P08482)	Rec	460	7
113.	Muscarinic acetylcholine receptor M4	ACM4_RAT (P08485)	Rec	478	7
114.	Neuronal acetylcholine receptor protein, alpha-5 chain precursor	ACH5_RAT (P20420)	Rec	452	4
115.	Potassium-transporting ATPase alpha chain (EC 3.6.1.36) (proton pump) (gastric H$^+$/K$^+$ ATPase)	ATHA_RAT (P09626)	Atp / Ppm	1033	8
116.	T-cell surface glycoprotein CD3 delta chain precursor (T-cell receptor T3 delta chain)	CD3D_RAT (P19377)	Rec	173	1
117.	Alpha-1B adrenergic receptor	A1AB_MESAU (P18841)	Rec	515	7
118.	Uncoupler protein	Hamster[10]	Tra / Ppm	304	7
119.	Calcium-transporting ATPase endoplasmic reticulum type (EC 3.6.1.38), class 2 (SERCA2)	ATCE_RABIT (P20647)	Atp / Npm	1042	8
120.	Calcium-transporting ATPase sarcoplasmic reticulum type (EC 3.6.1.38), fast twitch skeletal muscle, adult isoform (calcium pump) (SERCA1)	ATCB_RABIT (P11719)	Atp / Npm	994	8
121.	Potassium-transporting ATPase beta chain (EC 3.6.1.36) (proton pump) (gastric H$^+$/K$^+$ ATPase)	ATHB_RABIT (P18597)	Atp / Ppm	291	1
122.	5-Hydroxytryptamine 1D receptor (5-HT-1D) (serotonin receptor)	5HTD_CANFA (P11614)	Rec	377	7
123.	Adenosine A1 receptor	AA1R_CANFA (P11616)	Rec	326	7
124.	Adenosine A2 receptor	AA2R_CANFA (P11617)	Rec	412	7
125.	Sodium/calcium exchanger precursor (Na$^+$/Ca^{2+}-exchange protein)	NACA_CANFA (P23685)	Atp / Npm	970	11
126.	Alpha-1C adrenergic receptor	A1AC_BOVIN (P18130)	Rec	466	7
127.	Atrial natriuretic peptide clearance receptor precursor (ANP-C) (ANPRC)	ANPC_BOVIN (P10730)	Rec	537	1
128.	Butyrophilin precursor (BT)	BUTY_BOVIN (P18892)	Rec	526	1
129.	Cytochrome B561	C561_BOVIN (P10897)	Rdx / Ppm	273	6
130.	Endothelin B receptor precursor (ET-B) (endothelin receptor non-selective type)	ET1B_BOVIN (P28088)	Rec	441	7
131.	Type-1 angiotensin II receptor (AT1)	AG2R_BOVIN (P25104)	Rec	359	7
132.	Sodium/potassium-transporting ATPase alpha-1 chain (EC 3.6.1.37) (sodium pump) (Na$^+$/K$^+$ ATPASE)	ATN1_PIG (P05024)	Atp/Npm/Rbc	1021	8
133.	Cytochrome B6 complex (spinach chloroplast)	Spinach chloroplast[3]	Rdx / Ppm	211	5

Contd....

Appendix Contd....

134.	Diacylglycerol cholinephosphotransferase (EC 2.7.8.2) (SN-1,2-diacylglycerol cholinephosphotransferase) (CHOPT)	CPT1_YEAST (P17898)	Rec	407	7
135.	Ethanolaminephosphotransferase (EC 2.7.8.1) (ETHPT)	EPT1_YEAST (P22140)	Rec	391	7
136.	High-affinity glucose transporter HXT2	HXT2_YEAST (P23585)	Tra / Npm	541	12
137.	High-affinity glucose transporter HXT2	HXT2_YEAST (P23585)	Rec	541	12
138.	Inorganic phosphate transporter PHO84	PH84_YEAST (P25297)	Tra / Npm	596	12
139.	Plasma membrane ATPase 1 (EC 3.6.1.35)	ATH1_YEAST (P05030)	Atp / Npm	918	8
140.	Plasma membrane ATPase 2 (EC 3.6.1.35)	ATH2_YEAST (P19657)	Atp / Npm	947	8
141.	Cytochrome B	Monomeric yeast[12]	Rdx / Ppm	385	8
142.	CYO A (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	316	2
143.	CYO B (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	663	15
144.	CYO C (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	204	5
145.	CYO D (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	109	3
146.	CYO E (cytochrome O terminal oxidase complex)	*E. coli*[13]	Rdx / Ppm	296	7
147.	EF0 ATPase A subunit	*E. coli*[11]	Atp / Npm	83	2
148.	EF0 ATPase C subunit	*E. coli*[11]	Atp / Npm	79	2
149.	LAC permease	*E. coli*[8]	Tra / Ppm	417	12
150.	Light harvesting complex (LHC IIB polypeptide)	*Rb. sphaeroides*[6]	Rdx / Ppm	233	3
151.	Mitochondrial cytochrome C oxidase (subunit I)	*Rb. sphaeroides*[2]	Rdx / Ppm	565	12
152.	Mitochondrial cytochrome C oxidase (subunit III)	*Rb. sphaeroides*[2]	Rdx / Ppm	261	7
153.	Photosynthetic (light) reaction centre (H subunit)	*Rb. sphaeroides*[5]	Rdx / Ppm	262	1
154.	Photosynthetic (light) reaction centre (L subunit)	*Rb. sphaeroides*[5]	Rdx / Ppm	309	5
155.	Photosynthetic (light) reaction centre (M subunit)	*Rb. sphaeroides*[5]	Rdx / Ppm	282	5
156.	Cytochrome B/C1 complex	*Rhodobacter capsulatus*[4]	Rdx / Ppm	436	9
157.	Bacterial rhodopsin	*E. coli*[7]	Tra / Ppm	211	5

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Amino acid doublets and triplets in protein sequences – A database analysis

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Sequence analysis on the aspect of amino acid doublet and triplet repeats in 36,000 proteins comprising a total of 1,24,96,420 amino acid entries was carried out to study the structural aspects of proteins. Any deviation (normalized) of the doublet and triplet occurrence from their anticipated occurrence based on the frequency of the individual amino acid entries was attributed either to preferential selection (+ve deviation) or to non preferential selection (–ve deviation). All the homodoublets and homotriplets, except Ile–Ile–Ile, show positive deviation, implying the preferential selection for homomers. Most of the heterodoublets show marginal deviation indicating the expected distribution. A few doublets do show high positive and high negative deviations, containing the less propensity amino acids in the repeat. Most of the triplets which contain two identical amino acids in the repeat show positive deviations. In this category too, the high positively deviated triplets mainly contain less propensity amino acids, substantiating the economical usage of less propensity amino acids. A few triplets which contain two proline residues have large difference in observed and expected frequencies, in addition to high positive deviation indicating important structural role of proline residues. Most of the heterotriplets which have three different amino acid entries in the repeat show marginal deviations. The triplets with large deviation in this category also are dominated by less propensity amino acids. High frequency doublet pairs containing high propensity amino acid in the repeat (Glu–Lys, Lys–Glu), contribute structurally to α-helical conformation.

THREE-dimensional structural information (3-D information) about protein is buried in the one-dimensional amino acid sequences (1-D information). The number of available protein sequences is much larger than that of known three-dimensional structures. These protein sequences are available in SWISSPROT database. The three-dimensional structural information about the proteins is stored in the Brookhaven Protein Data Bank (PDB). These data banks contain valuable information on the structure of proteins. Exploiting these databases for information, regarding aspects of protein structure, is an integral part of research in structural biology[1–6]. Such analysis can be in aspects such as sequence analysis, protein fold investigation, secondary structure prediction studies, structure comparisons and knowledge-based model building of proteins[7–11]. This paper deals with the analysis of amino acid doublets and triplets in protein sequences and their implications to protein structure.

For sequence analysis the SWISSPROT Protein Sequence, Release 28 March 94 database was used. 36,000 proteins containing a total of 1,24,96,420 amino acid entries were considered for calculation.

The probability of occurrence of individual amino acid was calculated as

$$P(A) = \Sigma N_i\,(A)/\Sigma T_i,$$

where $N_i(A)$ is the number of entries for A in protein i, and T_i is the total number of amino acid entries in the corresponding protein.

The probability of occurrence of amino acid doublet repeat in the whole database was calculated as

$$P_{\text{comp}}(AB) = \Sigma\, N_i(AB)/\Sigma\,(T_i - 1),$$

where $N_i(AB)$ is the number of amino acid doublet entries AB in the protein i.

The probability occurrence of amino acid triplets in datum was calculated as

$$P_{\text{comp}}\,(ABC) = \Sigma N_i(ABC)/\Sigma\,(T_i - 2).$$

Theoretical estimates for the probabilities of amino acid doublets and triplets distribution were calculated using the following,

$$P_{\text{Theo}}\,(AB) = P(A)*P(B) = P_{\text{Theo}}(BA),$$

$$P_{\text{Theo}}\,(ABC) = P(A)*P(B)*P(C) = P_{\text{Theo}}(XYZ),$$

where X, Y, Z can be A or B or C. Expected frequency of occurrence (theoretical count) of doublet repeat AB is then $= P(A)*P(B)\Sigma_i\,(T_i{-}1)$. Expected frequency of occurrence (theoretical count) of triplet repeat ABC is then $= P(A)*P(B)*P(C)\,\Sigma_i\,(T_i{-}2)$.

The difference in count between the observed frequency (computed count) and the theoretical frequency (expected count) is normalized as follows:

For amino acid doublets

$$PD(AB) = ([P_{\text{comb}}(AB) - P_{\text{Theo}}\,(AB)]*100)/P_{\text{Theo}}\,(AB).$$

For amino acid triplets

$$PD(ABC) = ([P_{\text{comp}}(ABC) - P_{\text{Theo}}\,(ABC)]*100)/P_{\text{Theo}}(ABC).$$

The magnitude of the percentage of deviation could be treated as an index for the significance of observation.

The positive deviation is an indication of the preferential selection and the negative deviation is an indication of the non preferential selection of this repeat. In general, high percentage of deviation on the positive scale is likely to be an indication of an important role in the three-dimensional structure of proteins. On the other

Table 1. Probability of occurence of individual amino acids

Amino acid	Probability * 100
Leu	9.2
Ala	7.7
Ser	7.2
Gly	7.0
Val	6.5
Glu	6.3
Lys	5.8
Thr	5.8
Ile	5.6
Asp	5.3
Arg	5.3
Pro	5.0
Asn	4.5
Gln	4.0
Phe	4.0
Tyr	3.2
Met	2.4
His	2.2
Cys	1.8
Trp	1.3

Table 2. Deviation of homodoublets

Homo-doublets	Computed counts	Expected counts	Difference in counts	Deviation in %
His–His	10579	6330	4249	67
Cys–Cys	6517	3938	2579	66
Gln–Gln	31039	20211	13017	65
Glu–Glu	68819	48945	19875	41
Arg–Arg	47013	34167	12847	38
Trp–Trp	2879	2094	785	38
Ala–Ala	98462	72457	26005	36
Pro–Pro	42004	31375	10629	34
Lys–Lys	56521	42366	14155	33
Ser–Ser	82625	63424	19202	30
Asn–Asn	31687	24958	6728	27
Tyr–Tyr	16136	12934	3202	25
Met–Met	8560	6978	1583	23
Gly–Gly	73380	60832	12548	21
Thr–Thr	48197	42266	5931	14
Phe–Phe	22765	20061	2704	14
Asp–Asp	39288	34777	4511	13
Val–Val	58888	53057	5831	11
Leu–Leu	115209	105664	9548	9
Ile–Ile	41655	38951	2704	7

hand, repeats showing high negative deviation (doublet or triplet) may be detramental to protein structure.

The probability distribution of individual amino acids in the selected proteins is shown in Table 1. The results are in agreement with our earlier result[9], where the analysis was confined to a smaller database. As far as the individual amino acid occurrence is concerned, leucine occupies the highest position and tryptophan the last. Most of the amino acid entries have the probability distribution of more than 0.05 (mean probability distribution) with some exceptions asparagine, glutamine, phenylalanine, tyrosine, methionine, histidine, cysteine and tryptophan have probability of occurrence less than 0.05 while all other amino acids occur more frequently. Amino acids which have aromatic group in their side chain fall under the category of less propensity amino acids.

The computed and the theoretical probability distribution of the 400 amino acid doublets is shown in Figure 1. Figure 1 indicates that the computed probability of homomers always overshoots the theoretical, implying preferential selection of homomers. The range of deviation of homodimers varies from 7% to 67% (Table 2). This table and subsequent ones list the computed counts (actual counts), expected counts (theoretical counts) and the deviation in counts along with the percentage of deviation (normalized). The first three doublets showing more than anticipated frequency correspond to the less propensity amino acids histidine, cysteine and glutamine. The percentage of deviation was marginal (in between –5% and +5%) for most of the heterodoublets. But few heterodoublets have deviation ranging from +15% to +25% on the positive side, and –15% to –24% on the negative side. These heterodoublets are listed in Tables 3 *a* and 3 *b*. A roughly similar calculation was carried out by Rani *et al.*[11]. They have suggested that pair preference might be important for the three-dimensional structure of proteins. In the heterodoublets which show high positive deviation, one or both of the amino acids fall in the category of less propensity amino acids. The pairs that deviate from this rule are Glu–Lys and Lys–Glu ($P(\mathrm{Glu}) = 0.063$ and $P(\mathrm{Lys}) = 0.058$). A preliminary investigation on the conformational properties of these doublets was worked out by analysing the Ramachandran (Φ, Ψ) angles, (for 72 Glu–Lys repeats in 46 protein structures and 69 Lys–Glu repeats in 50 proteins). In Lys–Glu repeat, 72% and 66% of Ramachandran angles for Lys and Glu respectively are in the helical region. Similarly, in the Glu–Lys pair 71% for Glu and 63% for Lys are in helical region. Hence, these heterodoublets play a significant role in the three-dimensional structure of proteins especially in the α-helical segments. A (Φ, Ψ) distribution map related to these pairs is shown in Figures 2 and 3. In the doublets with high negative deviation also (Table 3 *b*), one or both of the amino acids of the repeats fall in the category of less propensity amino acid with exceptions being Glu–Pro, Glu–Ser and Pro–Ile. These doublet repeats have highest count difference in the negatively deviated heterodoublets. The reason for the deviated heterodoublets dominated by the less propensity amino acids may be due to their least occurrence, nature has used them economically by aligning them in proteins in a preferential (positive deviation) or in a non-preferential (negative deviation) way more than the average.

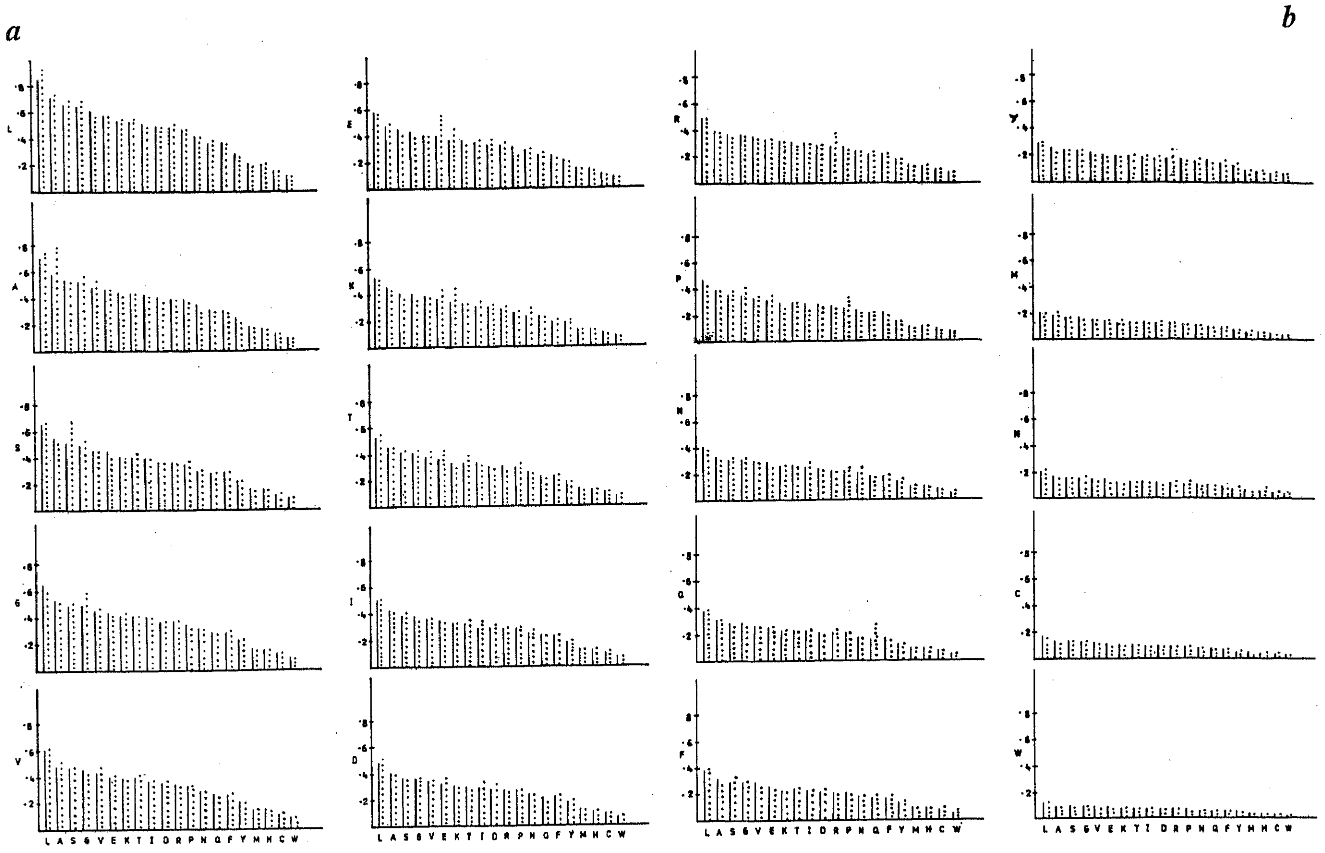

Figure 1. Theoretical (——) and computational (...) probability distributions. *a*, For amino acids Leu, Ala, Ser, Gly, Val, Glu, Lys, Thr, Ile, Asp. *b*, For amino acids Arg, Pro, Asn, Gln, Phe, Tyr, Met, His, Cys, Trp.

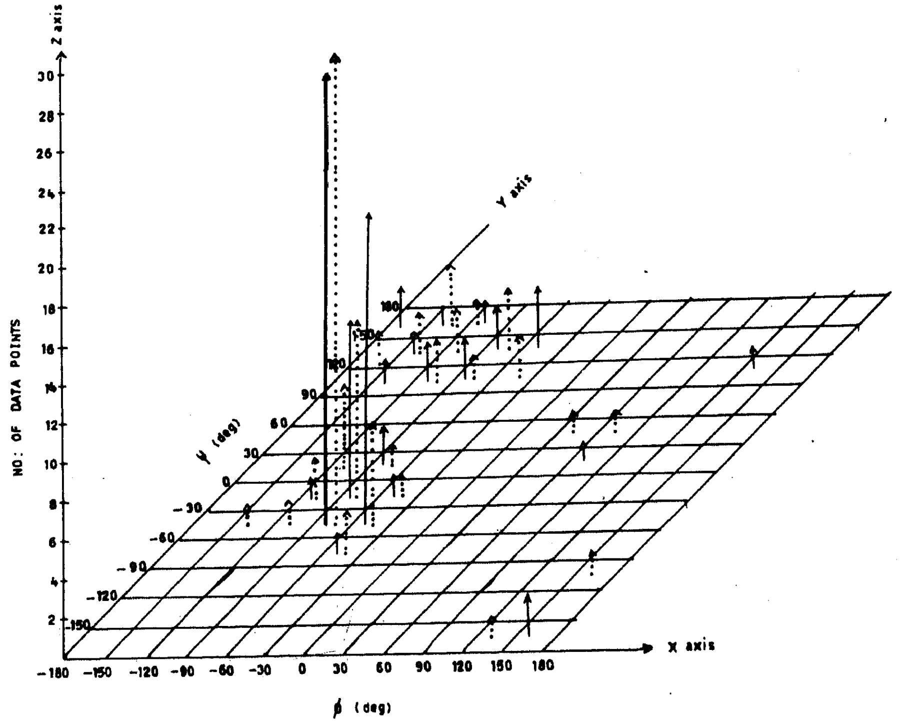

Figure 2. Ramachandran's (Φ, Ψ) angle distribution for Glu–Lys. ——, for Glu; ....., for Lys.

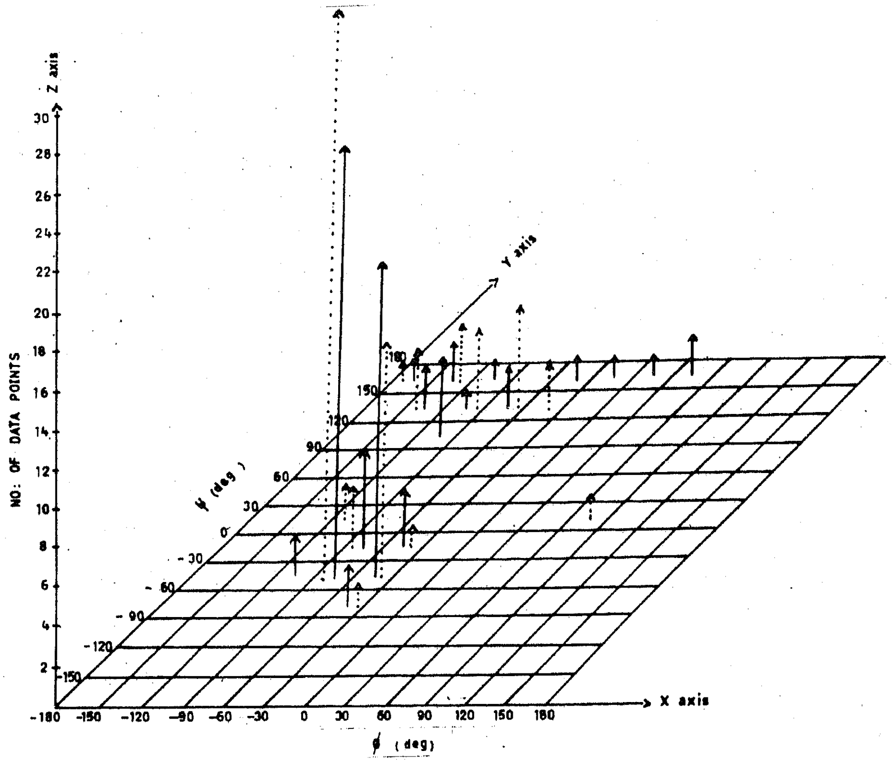

Figure 3. Ramachandran's (Φ, Ψ) angle distribution for Lys-Glu. ——, for Lys; ....., for Glu.

Table 3 *a*. Heterodoublets having positive deviation

Hetero-doublets	Computed counts	Expected counts	Difference in counts	Deviation in %
Cys–His	6243	4997	1246	25
His–Cys	6193	4997	1196	24
Glu–Lys	55275	45543	9732	21
Cys–Tyr	7564	7140	424	20
His–Pro	16946	14093	2853	20
Tyr–Cys	8523	7140	1383	19
His–Phe	13345	11264	2080	19
Met–Ala	26191	22491	3701	17
Lys–Glu	53181	45543	7638	17
Tyr–Trp	6056	5209	847	16
Tyr–Phe	18541	16111	2430	15
Asp–Tyr	24472	21208	3265	15
His–Trp	4187	3639	548	15

Table 3 *b*. Heterodoublets having negative deviation

Hetero-doublets	Computed counts	Expected counts	Difference in counts	Deviation in %
Trp–Pro	6156	8112	–1956	–24
Glu–Pro	30042	39188	–9146	–23
Pro–Met	11364	14803	–3439	–23
Cys–Met	4087	5246	–1159	–22
Glu–Ser	44733	55711	–10978	–19
Tyr–Ala	25070	30615	–5545	–18
His–Asp	12149	14840	–2692	–18
His–Glu	14641	17607	–2965	–17
His–Lys	13582	16386	–2804	–17
Asp–Gln	22192	26516	–4323	–16
Pro–Ile	29643	34952	–5308	–15

Table 4. Deviation of homotriplets

Homo-triplets	Computed counts	Expected counts	Difference in counts	Deviation in %
His–His–His	1089	143	946	662
Gln–Gln–Gln	6089	811	5278	651
Pro–Pro–Pro	5876	1569	4307	274
Cys–Cys–Cys	241	70	171	246
Arg–Arg–Arg	5503	1784	3719	208
Asn–Asn–Asn	3297	1113	2184	196
Glu–Glu–Glu	8727	3059	5668	185
Ser–Ser–Ser	11588	4513	7075	157
Ala–Ala–Ala	13616	5509	8107	147
Gly–Gly–Gly	9932	4238	5694	134
Trp–Trp–Trp	58	27	31	114
Met–Met–Met	353	165	188	114
Lys–Lys–Lys	5229	2464	2765	112
Asp–Asp–Asp	3779	1831	1948	106
Thr–Thr–Thr	4715	2455	2260	92
Tyr–Tyr–Tyr	764	416	348	84
Phe–Phe–Phe	1179	803	376	47
Val–Val–Val	4748	3452	1296	39
Leu–Leu–Leu	13507	9702	3805	38
Ile–Ile–Ile	2166	2172	–6	–0.28

When these studies were extended to triplet distribution (possible combination of 8000 triplets), all the homotriplets except, Ile–Ile–Ile, occurred with increased frequency, indicating a preferential selection of homomers (Table 4). For the homotriplets the percentage of deviation varies from –0.28 to 662. The highly deviated homotriplet is His–His–His and the least one is

Table 5. Deviation of triplet repeat which contains two identical amino acids in it

Triplet repeat with two identical amino acids	Computed counts	Expected counts	Difference in counts	Deviation in %
Cys–Trp–Cys	152	51	101	198
His–His–Pro	849	280	569	168
Cys–His–Cys	222	88	134	152
Cys–Ser–Cys	693	281	412	147
Cys–Cys–His	216	88	128	145
Tyr–Tyr–Cys	557	230	327	142
Cys–Cys–Phe	321	158	163	140
Cys–Tyr–Cys	302	127	175	138
Cys–Cys–Pro	452	198	254	127
Trp–Trp–Asn	211	93	118	127
Cys–Asn–Cys	387	176	211	119
Cys–Cys–Ser	611	281	330	118
His–His–Gln	548	255	293	115
His–Ile–His	755	353	402	114
Cys–Lys–Cys	489	230	259	113
Cys–Ala–Cys	634	299	335	112
Cys–Cys–Tyr	267	127	140	111
Pro–Pro–Gly	4584	2181	2403	110
His–Pro–His	650	317	333	109
Cys–Arg–Cys	430	206	224	108
Gln–Gln–His	945	455	490	108
Cys–Val–Cys	534	257	277	108
His–His–Arg	683	331	352	107
Cys–Gln–Cys	324	158	166	106
Cys–Asp–Cys	424	208	216	104
Cys–Thr–Cys	450	229	221	97
Gln–Gln–Pro	1964	1011	953	94
Cys–Cys–Arg	388	206	182	88
Cys–Cys–Gly	514	275	239	87
Pro–Gly–Pro	4042	2186	1856	85
Gln–Gln–Leu	3394	1856	1538	83
His–His–Met	272	150	122	83
Cys–Cys–Asn	321	176	145	82
Cys–Cys–Gln	285	158	127	80
His–Trp–His	148	82	66	80
Tyr–Tyr–Ser	1658	921	737	80
Trp–Trp–His	83	47	36	76
Cys–Ile–Cys	386	220	166	76
His–His–Phe	445	254	191	76
Arg–Trp–Arg	772	442	330	74
Pro–Thr–Pro	3170	1821	1349	74
Lys–Tyr–Lys	4595	2648	1947	74
His–Gln–His	441	254	187	73
Asn–Pro–Asn	2138	1249	889	71
Gln–Gln–Met	820	477	343	71
Trp–Pro–Trp	178	104	74	70
Glu–Glu–Met	1963	1156	807	70
Pro–Pro–His	1197	706	491	70
Gln–Pro–Gln	1695	1011	684	68
His–Phe–His	424	254	170	67
Asn–Asn–Met	978	589	389	66
Trp–Asn–Trp	154	93	61	65
Pro–Ala–Pro	3934	2386	1548	65
Pro–Asn–Pro	2310	1400	910	65
Pro–Ser–Pro	3638	2157	1481	63
Trp–Phe–Trp	136	83	53	63
His–His–Leu	943	582	361	62
His–His–Trp	133	82	51	62
Gln–Gln–Arg	1702	1056	647	61
Glu–Glu–Ile	4378	2728	1650	61
Asp–Asp–Glu	3481	2173	1308	60

Ile–Ile–Ile. Here the top two triplets which have a deviation of more than 600% are occupied by the less propensity amino acids, histidine and glutamine. It is also observed that most of the heterotriplets, which contain two identical amino acids in the repeat, always show increased frequency. Triplets which show deviation of more than 60% are listed in Table 5. In this group also (Table 5), the highly deviated triplets mostly contain one, two or three less propensity amino acids. A few exceptional triplets in this group are Pro–Pro–Gly (110%), Pro–Gly–Pro (85%), Pro–Thr–Pro (74%), Pro–Ala–Pro (65%), Pro–Ser–Pro (63%), Glu–Glu–Ile (61%) and Asp–Asp–Glu (60%). The above triplets, except Glu–Glu–Ile and Asp–Asp–Glu, contain proline in the repeat suggesting the importance of proline in the protein structures. The triplets which have different amino acids in the repeat invariably have marginal deviations (within + or –10%). A few triplets have deviations ranging from 60% to 196% on the positive side and –40% to –55% on the negative side (Table 6 and Table 7). All except four triplets showing positive deviation, contain two less propensity amino acids. This

Table 6. Heterotriplets having positive deviation

Heterotriplets	Computed counts	Expected counts	Difference in counts	Deviation in %
Trp–His–Met	254	86	168	196
His–Trp–Tyr	284	117	167	144
His–Pro–Trp	442	183	259	142
Arg–Cys–Trp	354	150	204	136
His–Tyr–Cys	376	160	216	135
Cys–Tyr-His	349	160	189	118
Tyr–Thr–Cys	824	415	409	99
His–Cys–Asp	523	263	260	99
Ile–Gly–Glu	5893	3042	2851	94
Tyr–Cys–Asn	612	319	293	92
Cys–His–Thr	555	291	236	91
Tyr–Pro–Trp	500	261	239	91
Tyr–Cys–Arg	706	373	333	89
Trp–Cys–Tyr	173	92	81	88
Cys–Gly–Lys	1635	901	734	82
Cys–Trp–Ala	396	219	177	81
Arg–Tyr–Trp	490	272	218	80
Trp–Tyr–Gln	376	209	167	80
Ile–Trp–His	365	203	162	80
Trp–Phe–Gln	466	261	205	79
Tyr–Trp–Asp	468	275	193	71
Cys–Trp–Met	115	68	47	69
Glu–Tyr–Trp	551	326	225	69
His–Pro–Glu	1476	881	595	68
Thr–Cys–Trp	278	167	111	67
His–Val–Trp	394	237	157	66
His–Pro–Asp	1236	743	493	66
Tyr–His–Cys	266	160	106	66
His–Phe–Asp	978	594	384	65
Met–Arg–Trp	329	200	129	65
Gln–Met–Trp	254	154	100	65
Tyr–Glu–Cys	734	446	288	64
Val–Trp–His	384	237	147	62

Table 7. Heterotriplets having negative deviation

Hetero-triplets	Computed counts	Expected counts	Difference in counts	Deviation in %
Trp–Pro–Met	85	191	–106	–55
Cys–Asp–Trp	75	152	–77	–51
Lys–Trp–Pro	232	471	–239	–50
Pro–Met–Arg	398	773	–375	–49
Glu–Pro–Asn	903	1748	–845	–48
Ala–Met–Trp	153	291	–138	–47
Trp–Cys–Met	36	68	–32	–47
Met–Trp–Pro	101	191	–90	–47
Phe–Lys–Trp	204	377	–173	–46
Glu–Trp–Pro	273	507	–234	–46
Cys–Glu–Met	179	328	–149	–46
His–Glu–Pro	482	881	–399	–45
Trp–Ser–Met	152	272	–120	–44
Gly–Met–Trp	153	266	–113	–43
Arg–Met–Trp	114	200	–86	–43
Pro–Met–Lys	493	861	–368	–43
Glu–Pro–Asp	1190	2063	–873	–42
Trp–Pro–Cys	83	144	–61	–42
Met–Cys–Pro	153	262	–109	–42
Pro–Met–Trp	113	191	–78	–41
Glu–Pro–Met	548	924	–376	–41
Ile–Trp–Pro	267	452	–185	–40
Phe–Lys–Met	410	768	–358	–40
Trp–Pro–Ile	269	452	–183	–40

also substantiates our earlier interpretation that nature has used the least occurring amino acids in an economical way by aligning them in proteins in a preferential or in a non-preferential way. The triplets Cys–Gly–Lys, His–Pro–Glu, His–Pro–Asp, contain two high propensity amino acids. In the triplets His–Pro–Glu, His–Pro–Asp, there may be a possibility of hydrogen bonding interaction between the side chain of histidine and the aspartic acid or glutamic acid. The triplet which has a deviation of 94% containing three high propensity amino acid is Ile–Gly–Glu, and is the only listed high positive deviated triplet having high count difference. High negative deviated heterotrimeric triplets having deviation ranging from –40% to –55% are listed in Table 7. Most of the high deviated triplets in the repeats have one, two or three less propensity amino acid except Glu–Pro–Asp.

In general, all the homodoublets and homotriplet repeats (except Ile–Ile–Ile) show positive deviation implying preferential selection. Most of the heterodoublets show marginal percentage of deviation (±5%). High deviated (+ve) heterodoublets except Glu–Lys and Lys–Glu mainly contain less propensity amino acids, indicating that nature has used the less propensity amino acids, in a preferential (+ve deviation) manner or in a non preferential (–ve deviation) manner in proteins. In the heterotriplets which contain two identical amino acids, most of the repeats show positive deviation and most of the high positive deviated triplets in this group contain one, two or three less propensity amino acids in the repeat, substantiating the economical usage of low propensity amino acids. A few triplets in this category contain two proline residues which have high difference in counts along with high positive deviation, indicating that proline plays an important role in the structure of proteins. Heterotriplets which contain different amino acids show marginal deviation (±10%). Most of the high deviated triplets in this category contain at least two less propensity amino acids. In the triplets His–Pro–Glu and His–Pro–Asp which contain two high propensity amino acids with high positive deviation (Table 6), we expect a structural role in the form of hydrogen bonding interaction between the side chain histidine and the glutamic acid or aspartic acid. In the high deviated heterodoublet Glu–Lys and Lys–Glu pair, more than 60% of the Ramachandran (Φ, Ψ) angles fall in the α-helical region, indicating the importance of these doublets in the α-helical structures in proteins. Discussions on the conformational aspect here have been restricted to Glu–Lys and Lys–Glu doublets. In depth analysis on the conformational significance of the highly deviated doublets and triplets and their role in the secondary structural elements of proteins will be considered in a later paper. As the database considered for the sequence analysis is very large (36,000 proteins containing a total amino acid 1,24,96,420), it is believed that multiple entries of families of proteins will not bias the result presented in this communication.

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Antimutagenic activity of tryptophan and alanine

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Antimutagenic activity of the amino acids tryptophan and alanine has been studied using ethyl methyl sulphonate and dimethyl sulphate-induced chromosomal aberration in onion root tip cells. Both the amino acids exhibited considerable antimutagenic activity by way of reducing the level of chromosomal aberrations. Duration of pre-treatment with the antimutagens was found to be important. On a comparative basis, tryptophan was found to be more efficient than alanine.

EVER since Muller first discovered the mutagenic action of X-ray in 1927 followed by the discovery of chemical mutagens by Auerbach in 1944, a large number of chemical substances have been added to the list, many of which are routinely used in plant breeding programmes. Even unsuspecting agents like laser beams, viruses and drugs like streptomycin are known to be mutagenic[1]. According to a conservative estimate[2], about 70,000 man-made chemicals are in everyday use and over a thousand new chemicals are put to use every year. Therefore the list and concern over mutagenic substances is never-ending. Environmental mutagens have generally two broad category effects. Firstly, they cause toxicity to the organism exposed, which manifests in the form of various ailments to mortality. The other is a long-term effect – they cause mutations which are mostly deleterious and these are transmitted to the future generations who will suffer even if they are not exposed. Against this background, antimutagens which can neutralize the mutagenic effect, deserve serious attention. Particularly certain vitamins and pro-vitamins like α-tocopherol, ascorbic acid, β-carotene; amino acids like tryptophan and cystein, metal salt of selenium, etc. are reported to have considerable antimutagenic property[3–5]. Since the concept of antimutagen developed in the 1950s, about 200 compounds are known to have varied degree of antimutagenic property[3]. The potential use of antimutagen is envisaged in plant tissue culture where chromosomal aberration is very common and which is one of the major reasons for somaclonal variation. Antimutagens can also be used for long-term cryopreservation of seeds, embryo, somatic embryo, tissue, etc. to provide the much needed genetic stability. The present study was undertaken to investigate the antimutagenic potency of amino acids – tryptophan and alanine, using chromosomal aberration study as the criterion, with emphasis on duration of pre-treatment (Tables 1, 2).

For the present study, onion root tips were taken as the study material. The mutagens used were ethyl methane sulphonate (EMS) and dimethyl sulphate (DMS) at concentrations of 0.05%, 0.1%, 0.2% and 0.3%. For tryptophan and alanine, 500 ppm solutions were used. Healthy onions were rooted on moist sand for one day. Thereafter the roots were washed with distilled water

Table 1 *a*. Decline in chromosomal aberrations due to pre-treatment with tryptophan followed by EMS treatment

EMS (%)	Tryptophan pre-treatment (500 ppm)	Chromosomal aberration: Laggards	Breakages	Bridges	Total aberration (%)
0.00	Nil	0	0	0	0
0.05		0	0	6	6
0.10		0	0	8	8
0.20		0	0	9	9
0.30		0	1	11	12
0.00		0	0	0	0
0.05	10 h	0	0	2	2
0.10		0	0	4	4
0.20		0	0	5	5
0.30		0	0	5	5
0.00		0	0	0	0
0.05		0	0	0	0
0.10	20 h	0	0	0	0
0.20		0	0	0	0
0.30		0	0	1	1

Duration of EMS treatment = 8 h.
CD for tryptophan pre-treatment: At 5% probability level = 0.544, At 1% probability level = 0.733.

Table 1 *b*. Decline in chromosomal aberrations due to pre-treatment with tryptophan followed by DMS treatment

DMS (%)	Tryptophan pre-treatment (500 ppm)	Chromosomal aberration: Laggards	Breakages	Bridges	Total aberration (%)
0.00	Nil	0	0	0	0
0.05		0	0	4	4
0.10		0	0	5	5
0.20		0	0	7	7
0.30		0	0	8	8
0.00		0	0	0	0
0.05	10 h	0	0	1	1
0.10		0	0	2	2
0.20		0	0	3	3
0.30		0	0	3	3
0.00		0	0	0	0
0.05		0	0	0	0
0.10	20 h	0	0	0	0
0.20		0	0	1	1
0.30		0	0	1	1

Duration of DMS treatment = 8 h.
CD for tryptophan pre-treatment: At 5% probability level = 0.384, At 1% probability level = 0.517.

Table 2 *a*. Decline in chromosomal aberrations due to pre-treatment with alanine followed by EMS treatment

EMS (%)	Alanine pre-treatment (500 ppm)	Chromosomal aberration			Total aberration (%)
		Laggards	Breakages	Bridges	
0.00		0	0	0	0
0.05		0	0	6	6
0.10	Nil	0	0	8	8
0.20		0	0	9	9
0.30		0	1	11	12
0.00		0	0	0	0
0.05		0	0	3	3
0.10	10 h	0	1	4	5
0.20		0	0	5	5
0.30		0	0	6	6
0.00		0	0	0	0
0.05		0	0	1	1
0.10	20 h	0	0	2	2
0.20		0	0	2	2
0.30		0	0	3	3

Duration of EMS treatment = 8 h.

CD for alanine pre-treatment: At 5% probability level = 1.247, At 1% probability level = 1.680.

Table 2 *b*. Decline in chromosomal aberrations due to pre-treatment with alanine followed by DMS treatment

DMS (%)	Alanine pre-treatment (500 ppm)	Chromosomal aberration			Total aberration (%)
		Laggards	Breakages	Bridges	
0.00		0	0	0	0
0.05		0	0	4	4
0.10	Nil	0	0	5	5
0.20		0	0	7	7
0.30		0	0	8	8
0.00		0	0	0	0
0.05		0	0	4	4
0.10	10 h	0	1	5	6
0.20		0	0	7	7
0.30		0	0	8	8
0.00		0	0	0	0
0.05		0	0	3	3
0.10	20 h	0	0	4	4
0.20		0	0	4	4
0.30		0	0	5	5

Duration of DMS treatment = 8 h.

C.D for alanine pre-treatment: At 5% probability level = 0.430, At 1% probability level = 0.579.

and treated with tryptophan and alanine solutions for 10 h and 20 h respectively. Subsequently the roots were again washed and treated with different concentrations of the mutagens for 8 h after which the roots were thoroughly washed in running tap water. The root tips were collected and fixed in aceto-alcohol for 2 h and then transferred to 70% ethanol. Root tip squash was made in aceto-carmine and then observed for chromosomal aberrations. For each treatment, 100 dividing cells were observed and each treatment was replicated thrice. The control sets were treated with distilled water followed by mutagenic treatment. The data were statistically analysed using Fisher's method for determination of variance ratio.

Chromosomal aberrations were found in the form of laggards, breakages and bridges in early anaphase stage. Tryptophan exhibited pronounced antimutagenic activity. In case of tryptophan pre-treatment for 10 h followed by EMS treatment, the range of aberration came down to 2–5% against 6–12% in control. But when the duration of pre-treatment was increased to 20 h, there was virtually no chromosomal aberration, except 1% aberration in case of 0.3% EMS treatment. The impact of duration of tryptophan pre-treatment has been found to be highly significant (CD = 0.733; $P > 0.01$). Tryptophan pre-treatment was similarly effective against DMS also. Pre-treatment with tryptophan for 10 h followed by DMS treatment resulted in chromosomal aberration 1–3% as against 4–8% in control. On increasing the pre-treatment duration to 20 h, there was no aberration at lower concentration of DMS treatment; only at higher concentration of DMS treatment 1% aberration was observed. The observation has been found to be highly significant (CD = 0.517; $P > 0.01$).

Alanine pre-treatment for 10 h followed by EMS treatment brought about nearly 50% reduction in chromosomal aberration. Increasing the duration of treatment to 20 h resulted in remarkable reduction in chromosomal aberration, when the range of aberration was 1–3% as against 6–12% in control. The effect of increasing duration of alanine pre-treatment on reducing chromosomal aberration has been found to be highly significant (CD = 1.68; $P > 0.01$). However, alanine pre-treatment for 10 h against DMS treatment failed to reduce chromosomal aberration. But a higher duration, i.e. 20 h pre-treatment of alanine was effective when the range of aberration was 3–5% as against 4–8% in control. Statistically it has been found to be highly significant (CD = 0.579; $P > 0.01$).

From the present study it is evident that tryptophan has remarkable antimutagenic activity by way of protecting the chromosomes against physical damage by mutagens. Moreover, another amino acid alanine, previously untested, has been found to have considerable antimutagenic activity although its efficacy is comparatively lesser than tryptophan. Cherkasov[6] studied the anti-mutagenic activity of tryptophan by treating the seeds of *Allium cepa* and found considerable antimutagenic activity. However, his study showed that variation in concentration of tryptophan made little difference. In the present study the emphasis was on duration of pre-treatment and the results clearly show that longer duration of pre-treatment, i.e. 20 h is remarkably effective. Moreover, tryptophan is equally effective against both the mutagens EMS and DMS.

Unlike tryptophan, alanine is not much effective against DMS, although it is considerably effective against EMS. But like tryptophan, in case of alanine also, the duration of pre-treatment made considerable difference. While a 10 h pre-treatment was simply ineffective against DMS, the same concentration at longer duration, i.e. 20 h pre-treatment could considerably reduce chromosomal aberration. Similar observations regarding greater efficacy of antimutagen following pre-treatment for longer duration like 20 h were reported by Abutalybov *et al.*[7] working with *Allium fistulam* using ionol and Alekperov[15] working with *Crepis capillaris* using α-tocopherol as antimutagen. It appears that different antimutagens are not equally effective against various mutagens. But an ideal antimutagen should be the one which is universally effective against all mutagens or at least a broad spectrum of mutagens. Tryptophan deserves further and detailed investigation to ascertain whether it can be called a broad spectrum antimutagen or not. Apart from a chromosomal aberration test in onion root tip, the efficacy of antimutagens has been tested in the prokaryotic system, viz. *Salmonella typhimurium* by Bala and Grover[8], Grover and Bala[9] using citrus and myroblan fruit juice as source of antimutagen which are known to be very rich in ascorbic acid. Antimutagenic activity of polyphenols like caffeic acid and gallic acid has been demonstrated by Chan *et al.*[10] using the Salmonella test system.

Apart from tryptophan and alanine, at least another amino acid, cystein is known to have antimutagenic effect as demonstrated by Garina and Nurzhanova[11] and Ionaitis Sokolov and Ranchyalis[12]. It is not understood how a simple amino acid molecule can protect the chromosomes against mutagenic treatment damage. Kada *et al.*[13] classified antimutagens into two major groups: desmutagen and bioantimutagen. Desmutagens are defined as those which inactivate mutagen *in vitro* before they reach the interior of cell. It is likely that antimutagens like tryptophan and alanine neutralize the free radicals generated by the mutagens, thereby rendering them ineffective. Strelchk[14] detected antimutagenic activity of the extracts of *Eleutherococcus senticosum* against EMS by post treatment. However the extract had a protective effect only in the first four hours after post treatment. Obviously the mutagens do all the damage in the first four hours after treatment and therefore post treatment with antimutagen beyond this period is of no avail.

The present study clearly shows that apart from tryptophan, another amino acid alanine also has antimutagenic property, although tryptophan is superior to alanine as antimutagen.

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Anti-staphylococcal activity of *Pseudomonas aeruginosa*

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Anti-staphylococcal activity of *Pseudomonas aeruginosa* was studied on a total of 118 strains of staphylococci. The results revealed the existence of a highly active anti-staphylococcal *Pseudomonas aeruginosa* metabolite which appears to be pyocyanine. It was even active against methicillin resistant *Staphylococcus aureus* (MRSA) and coagulase negative staphylococci (CONS).

PSEUDOMONAS represents the major group of non-differentiating microorganisms producing antibiotics[1]. The practical use of antibiotics from *Pseudomonas* sp. dates back to the period before the antibiotic era. Emerich and Low[2] reported that the cell-free culture fluid of *Pseudomonas aeruginosa*, concentrated to one tenth of its original volume, killed several kinds of bacteria. This was extensively used in the therapy of diphtheria, influenza and meningitis during the first two decades of the century[1]. During the antibiotic era, approximately 50 different antibiotic substances from *Pseudomonas* sp. were discovered. Of these compounds, pyocyanine and pyrrolnitrin were most abundantly present. We failed to find in literature any study regarding anti-staphylococcal activity of *P. aeruginosa*, except a report on the biosynthesis of batumin[3], especially on methicillin-resistant *Staphylococcus aureus* (MRSA) and coagulase negative staphylococci (CONS).

Methicillin-resistant *Staphylococcus aureus* (MRSA) emerged in 1980 as a major clinical and epidemiologic problem in hospitals[4]. It was suggested that hospitals of all sizes were facing the MRSA problem[5]. The coagulase negative *Staphylococcus* sp. (CONS) as a group constitutes a major component of the normal microflora of the human. Over the last two decades, there has been an increase in the documentation of infections due to CONS, especially in hospitalized immunocompromised patients and in patients having prosthetic and indwelling devices[5].

In this study, we evaluated 103 strains of *S. aureus* and 15 strains of coagulase negative staphylococci for their susceptibility to metabolites of *P. aeruginosa* by testing against 80 *Pseudomonas* strains. In addition, attempts were also made to identify and characterize the active metabolites of *P. aeruginosa*.

A total of 80 *P. aeruginosa* strains isolated from a variety of clinical specimens were identified by conventional tests described elsewhere[6]. Of these 80 strains, 75 were pyocyanine producers and 5 were non-pyocyanine producers. The strains were maintained in nutrient agar slants and stored at 4°C and subcultured once in every 15 days.

A total of 103 strains of *S. aureus*, including 70 methicillin sensitive *S. aureus* (MSSA), 30 methicillin resistant *S. aureus* (MRSA) isolated from a variety of clinical specimens, one strain each *S. aureus* NCTC 6571, *S. aureus* Cowan I and *S. aureus* Plazen were maintained in soft nutrient agar butts at 4°C. The methicillin susceptibility of these strains was tested by the methods described elsewhere[7]. Fifteen strains of coagulase negative *Staphylococcus* sp. isolated from a variety of clinical specimens, were maintained in soft nutrient agar butts.

Anti-staphylococcal activity of *P. aeruginosa* was tested using (i) pyocin typing like method and (ii) agar overlay method.

In pyocin typing like method, a young peptone water culture of *P. aeruginosa* adjusted to 0.5 McFarland turbidity standard was inoculated in the centre of a dry nutrient agar plate as a line of 4 mm width using a bacteriological loop. The plates were incubated at 37°C for 18–24 h. Then the growth was gently scrapped with an 'L' shaped thin glass rod. The remaining organisms were spread over the agar surface with a cotton swab soaked in saline. The plates were exposed to UV light in a micro-titre plate sterilizer for 5 min. (ref. 8). After killing *Pseudomonas*, young peptone water culture of staphylococci, adjusted to 0.5 McFarland standard, was inoculated as 4 mm width lines at right angles to the original *Pseudomonas* inoculum. The plates were incubated at 37°C for 18–24 h and scanned for inhibition of growth of staphylococci over original *Pseudomonas* inoculum[8].

In the agar overlay method, 6 strains of *P. aeruginosa* were inoculated into dry nutrient agar (NA) plates (8 cm diameter) as spots by transferring 10 µl of inoculum prepared as explained above. The plates were incubated at 37°C for 18–24 h. The growth was scrapped and the plates were exposed to UV as described above. These plates were overlaid with 10 ml of sterile molten agar at 45–50°C to which 25 µl of staphylococcal inoculum was added and mixed. The plates were incubated at 37°C for 18–24 h and scanned for the zone of inhibition of growth over the *Pseudomonas* inoculum spots.

The effect of presence of oxygen on antibiosis was assessed by growing 10 pyocyanine-producing strains of *P. aeruginosa* on nutrient agar supplemented with 0.1% KNO_3 (ref. 9) and kept anaerobically in a commercially available gas pack system. The growth was scrapped and exposed to UV light and anti-staphylococcal activity was tested as described above.

The effect of iron in the medium on antibiosis was tested by incorporating iron ($FeCl_3$) at 50, 100, 150, 200, 250 and 300 mg/l separately into nutrient agar[10]. The anti-staphylococcal activity was assayed as described above.

With both the methods, all *S. aureus* strains including MRSA and CONS were found to be susceptible to pyocyanine produced by *P. aeruginosa*. The inhibition zone was directly proportional to the bluish-green discolouration on the plate. While none of the 5 strains of pyocyanine non-producing *P. aeruginosa* showed any activity on staphylococci, *P. aeruginosa* grown anaerobically failed to inhibit the growth of staphylococci, tested. Incorporation of iron into the medium did not affect the susceptibility of *S. aureus* to *P. aeruginosa*.

This study clearly shows the presence of some metabolite(s) of *P. aeruginosa* which is highly active against staphylococci. The most promising finding is that, all the 30 strains of MRSA tested were found to be susceptible. The possibility of depletion of nutrients by the *Pseudomonas* strains from the medium as a reason for its anti-staphylococcal activity is ruled out as the agar overlay method showed absolute agreement with the results of pyocin typing like method. Pyocyanine production showed a positive correlation with the anti-staphylococcal activity of *P. aeruginosa*. *P. aeruginosa* grown in the absence of oxygen failed to exhibit anti-staphylococcal activity. Since pyocyanine is an oxygen-dependent pigment, this observation suggests the role of pyocyanine in the anti-staphylococcal activity of *P. aeruginosa*. Kumar and Bezbaruah[10] reported that siderophose produced by the *Pseudomonas* had a vital role in its anti-fungal activity. Our studies on the other hand, showed that siderophose is not responsible for its anti-staphylococcal activity.

In conclusion, pyocyanine-producing *P. aeruginosa* exhibits potent anti-staphylococcal activity even on MRSA and CONS strains. The active substance appears to be pyocyanine. This may be exploited for the development of an effective anti-staphylococcal agent.

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Community structure of larval trematode fauna of the snail *Thiara tuberculata* from a freshwater stream at Visakhapatnam, Andhra Pradesh

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Monthly samples of the snail *Thiara tuberculata*, collected from a freshwater stream at Visakhapatnam, were examined for larval trematode infections. Cercarial infections occurred in 38.2% of the snails. Nineteen species of cercariae belonging to 10 families were found, with xiphidiocercariae as dominant members. Fifteen species of cercariae belonged to allogenic category and four species were autogenic. Four species are identified as having zoonotic potential with fish serving as second intermediate hosts. No seasonal pattern is exhibited in the overall prevalence of infection and the community diversity.

THE freshwater bodies of tropical countries harbour dense populations of snails that serve as intermediate hosts for a wide range of digenetic trematodes infecting various groups of vertebrates including man. Investigations dealing with analysis of larval trematode fauna of snails, their transmission patterns and host–parasite relationships are receiving considerable attention. From the Indian region, studies relating to larval trematode fauna of snails have received attention ever since the publication of the monumental work by Sewell[1]. The approach so far has been taxonomic in nature. Information available on ecological aspects of parasitism in snails is very scanty.

In recent studies, much emphasis has been given to investigations dealing with analysis of community structure of larval digenean infections in snails[2–8]. The community with reference to parasites has been defined as the complex assemblage of parasites occurring within a host. Terms such as infracommunity, component community and compound community are in use, to define various heirarchical levels, the infracommunity to include all the parasites in a single host, the component community including all the parasites in a single population of hosts and the component community denoting all the parasite communities within an ecosystem[2]. The parasite community within a host population is considered as complex and a result of continuous interaction of host–parasite and environment. Its species composition and diversity are under the control of a complex set of biotic and abiotic factors which have direct impact on the transmission patterns. An analysis of this relationship is expected to be useful in understanding the forces which operate in structuring the parasite communities.

In the course of our studies on larval trematode infections of freshwater snails of Visakhapatnam region, it was found that the snail *Thiara tuberculata* in a local freshwater stream serves as a suitable host for a wide range of larval digeneans. An investigation has, therefore been undertaken during 1992–1994, to study the organization of larval trematode fauna of the snail including the species composition, their diversity, dominance and seasonal changes.

The study area is a small freshwater stream originating from Mehadrigedda reservoir flowing in a north-easterly direction, to ultimately join the creek near a fishing harbour. The snail *T. tuberculata* forms dense populations in the steam throughout the year. The collection spot located adjacent to the reservoir is surrounded by mango trees on which nestle a number of resident birds like pond herons, egrets and kites. Ducks are also common. Domestic animals like sheep and cattle visit the area.

The presence of freshwater snakes was also occasionally noted.

The snails, totalling 3173 in number, were collected as monthly samples during the period October, 1992 to September, 1994 and each monthly sample contained 86 to 168 snails. The snails were isolated individually in petri dishes and infected snails were identified by examining the water for the presence of cercariae. The snails which were not emitting cercariae were crushed and digestive gland examined for the presence of cercarial infections. Data on the number of snails examined with each variety of cercariae were recorded for each monthly sample.

From the data collected, the overall prevalence of infection with larval trematodes, the prevalence of infections with individual species of cercariae, in the monthly samples were determined. The community structure of larval trematode fauna was analysed employing various indices that have been used by earlier workers[2,5,6]. The species richness, the dominance and the Shannon–Wiener's diversity index were used as indices of community diversity. Species richness was determined for each monthly sample. The Shannon–Wiener's diversity index was calculated using the formula

$$H' = -\Sigma(p_i \times Inp_i),$$

where p_i is the proportion of snails infected with each species of cercaria in the monthly sample.

The snail was found to host 19 species of cercariae distributed over 10 families (Table 1). Information on the prevalence of infection with various cercariae and their life cycle pattern is given in Table 2. Among the cercariae encountered, the xiphidiocercariae represented by seven species constituted the most dominant members of the cercarial fauna. Infections with cercariae of *Procerovum varium*, *Haplorchis* spp. and *Centrocestus formosanus* capable of infecting humans with fish as intermediate host, occurred in 11.91% of snails. Cercariae of *Transversotrema patialense, Philophthalmus nocturnus, Atrophecaecum burminis* and *Haplochoides mehrai* occurred fairly commonly. Infections with strigeid, schistosome and renicolid cercariae were rare.

The data reveal that 15 out of 19 cercariae belong to allogenic category with part of their life cycle occurring in terrestrial birds. The remaining four species are autogenic with life cycle operating in hosts that are confined to the stream. The data also reveal that of the 15 species using birds as definitive hosts, 6 species depend on piscine hosts for transmission, 2 species depend on aquatic insects and 1 species on molluscs. Cercariae of 4 species namely *H. pumilio*, *H. taichui*, *P. varium* and *C. formosanus*, have zoonotic potential with fish acting as second intermediate hosts.

Out of 3173 snails examined, 1213 individuals, i.e. 38.23% carried patent infections with larval trematodes. Among the 1213 infected snails, 28 carried double infections. The overall prevalence in the monthly samples ranged from a maximum of 46.97% during October 1992 to the lowest of 30.1% during March 1994 (Figure 1 *a*). The numbers of cercarial species in each sample varied from 10 to 15 with an average of 12 species (Figure 1 *b*). Both these parameters exhibited no pronounced seasonal fluctuations, indicating homogeneity in the infection state of snail population in the stream, over different months. Further evidence to the homogeneity of infections was provided by the values of Shannon–Wiener's diversity index which varied in the monthly samples from 2.1 to 2.5 with an average of 2.3 and showed no marked seasonal changes (Figure 1 *c*).

The study revealed the infection rate of the snail *T. tuberculata* with larval digeneans to be very high with overall prevalence of 38.23% and larval trematode assemblage comprising as many as 19 species. Both these parameters are far higher than those recorded for the snail from other localities in India[9–11] and abroad[12,13]. This indicates that the physico-chemical and biotic features of the local stream, offer ideal conditions for promoting infections in the snail and the locality thus constitutes a natural focus for helminth infections with the snail *T. tuberculata* playing a prominent role as a vector.

Table 1. List of cercariae collected from *Thiara tuberculata* from the stream

- Family: Strigeidae Railliet, 1919
  - *Cercaria Thiara I Swarnakumari*, 1992
- Family: Schistosomatidae Poche, 1907
  - *Cercaria Thiara* n.sp.
- Family: Transversotrematidae Yamaguti, 1953
  - Cercaria of *Transversotrema patialense* (Soparkar, 1924)
- Family: Philophthalmidae Travassos, 1918
  - Cercaria of *Philophthalmus nocturnus* Looss, 1907
- Family: Psilostomidae Looss, 1900
  - Cercaria of *Grysoma indica* Umadevi and Madhavi, 1995
- Family: Eumegacetidae Travassos, 1922
  - Cercaria of *Eumegacetes artamii* Mehra, 1935
  - Cercaria of *Eumegacetes* sp.
  - Cercaria of *Orthetrotrema monostomum*, Macy and Basch, 1972
  - *Cercaria Thiara III* Swarnakumari, 1992
  - *Cercaria Thiara IV* Swarnakumari, 1992
- Family: Lecithodendriidae Odhner, 1910
  - *Cercariae Indicae LXVII* Murty, 1976
  - *Cercaria Thiara V* Swarnakumari, 1992
- Family: Renicolidae Dollfus, 1939
  - *Cercariae Indicae XIV* Sewell, 1922
- Family: Heterophyidae Odhner, 1914
  - Cercaria of *Centrocestus formosanus* Nishigori, 1924
  - Cercaria of *Haplorchoides mehrai*, Pande and Shukla, 1976
  - Cercaria of *Haplorchis pumilio* (Looss, 1896)
  - Cercaria of *Haplorchis taichui* Nishigori
  - Cercaria of *Procerovum varium* Onji and Nishio, 1916
- Family: Acanthostomidae Poche, 1926
  - Cercaria of *Atrophecaecum burminis* Bhalerao, 1926

Table 2. Overall prevalence and life-cycle pattern for the guild of larval trematodes in the snail *Thiara tuberculata*

Trematode species	Prevalence (1992–94)	Definitive host	Life-cycle pattern	Category
Strigeid cercaria	0.13	Birds	Snail–fish–piscivorous birds	Allogenic
Schistosome cercaria	0.09	Birds	Snail–birds	Allogenic
Transversotrema patialense	2.45	Fish	Snail–fish (Progenetic)	Autogenic
Philophthalmus nocturnus	2.02	Birds	Snail–external–birds	Allogenic
Grysoma indica	0.47	Ducklings	Snail–snail–ducklings	Allogenic
Xiphidiocercariae	11.31			Allogenic
Cercaria of *Eumegacetes artamii*		Birds	Snail–aquatic insect–bird	Allogenic
Cercaria of *Eumegacetes* sp.		Birds	Snail–aquatic insect–bird	Allogenic
Cercaria of *Orthetrotrema monostomum*		Dragon fly naiads	Snail–dragonfly naiads	Autogenic
Cercaria Thiara III		Birds	–	Allogenic
Cercaria Thiara IV		Birds	–	Allogenic
Cercaria Thiara V		Birds	–	Allogenic
Cercariae Indicae LXVII		Birds	–	Allogenic
Renicolid Cercaria	0.28	Birds	Snail–fish–birds	Allogenic
Centrocestus formosanus	2.68	Birds	Snail–fish–birds	Allogenic
Haplorchoides mehrai	4.03	Birds	Snail–fish–fish	Autogenic
Haplorchis pumilio	3.34	Birds	Snail–fish–birds	Allogenic
H. taichui	2.43	Birds	Snail–fish–birds	Allogenic
Procerovum varium	3.46	Birds	Snail–fish–birds	Allogenic
Atrophecaecum burminis	6.40	Water snake	Snail–fish–snake	Autogenic

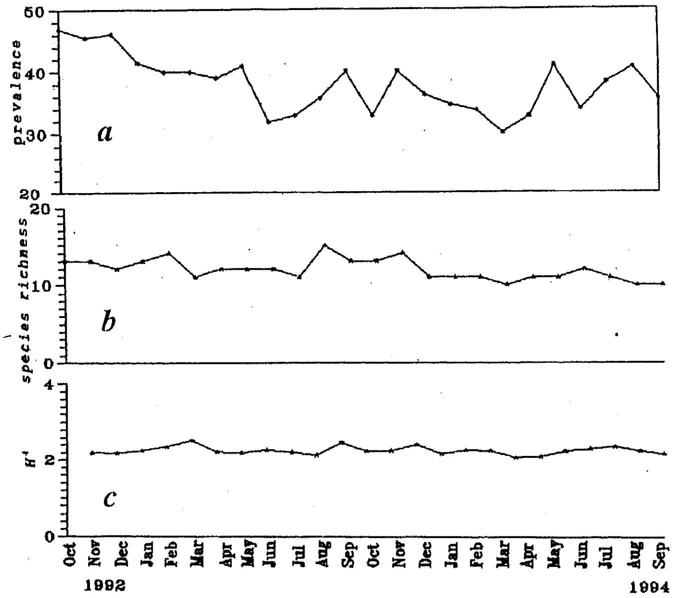

Figure 1. Seasonal changes in the community parameters of larval trematode infections in the snail *Thiara tuberculata*, from a freshwater stream at Visakhapatnam. *a*, Prevalence; *b*, Species richness; *c*, Shannon–Wiener's diversity index (*H*′).

The various larval digeneans infecting the snail exhibited a dominance heirarchy with xiphidiocercariae dominating the parasitic fauna. The dominance of xiphidiocercariae is evidently contributed by the abundant occurrence in the habitat of aquatic insects and insectivorous birds that serve as second intermediate and definitive hosts respectively. The influence of aquatic snakes on the cercarial fauna of the snail is also evident by the common occurrence of *Atrophecaecum burminis* cercariae the adults of which occur in the water snake *Natrix piscator*. The frequent occurrence of heterophyid cercariae seems to be caused by the presence in the stream, of several species of fish that serve as intermediate hosts. It is obvious from the present study that *T. tuberculata* plays a key role in the habitat in the establishment, operation and completion of a number of trematode life cycles. The lack of seasonality, in the overall prevalence of larval trematode infections in the locality is also in contrast with the situation prevailing in many other regions where seasonal fluctuations in the prevalence of infections in snails involving two peak levels were recorded[9,14–16]. Lack of seasonality is also exhibited in the diversity of parasite fauna. This seems to be caused by the interplay of several factors including homogeneity in the physico-chemical and biotic features of the stream, the resident nature of the birds, the favourable temperature of tropical climate and continuous availability of young snails that are highly susceptible to the infection.

The high prevalence noted with the four species of heterophyid cercariae having zoonotic potential deserves attention. Human infections with these species transmitted through consumption of fish have been recorded from south-east Asian countries including Philippines, Taiwan and Egypt[17–19]. Pande and Shukla[20] have earlier reported the zoonotic significance of heterophyid infections of Indian region and cautioned against consumption of raw fish.

A preliminary survey of fishes in the stream revealed fairly common occurrence of infections with metacercariae of *Procerovum* sp., *Haplorchis* spp. and *Centrocestus* sp. in the muscles and gills of *Channa* spp., *Gambusia* sp., *Puntius* sp. and *Oryzias* sp. Although some of these fish are consumed by local communities, no information is available on the prevalence of infections with heterophyid trematodes in the individuals. The situation, however, demands an in-depth epizootiological study involving a detailed analysis on a seasonal basis, the physico-chemical features of the environment, the prevalence of cercarial infections in the snails and metacercarial infections in the fishes, the role of birds as transmitting agents and the extent of human involvement. Moreover, the snail host in the stream with its broad spectrum of larval trematode infections offers a suitable model for the study of host–parasite–environment interactions.

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On the theory and utility of spectral seismograms

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A spectral seismogram enables a quick and detailed analysis of an earthquake waveform in terms of the frequency–time distribution of the radiated energy. In this approach, a broad-band signal is first filtered using various bandpass filters to obtain the corresponding bandpass seismograms. Each of these seismograms is then Hilbert transformed, to obtain the corresponding instantaneous amplitudes with respect to time, for each mid-frequency. The resulting amplitudes are then represented using a colour code or a grey scale to obtain a spectral seismogram. We discuss here, the theory of spectral seismograms and as examples, present the spectral seismograms of 3 earthquakes from the Burmese arc region.

AN earthquake source radiates energy over a wide range of frequencies in the form of longitudinal and shear waves. These waves, besides generating various surface waves, get reflected and refracted inside the earth, thereby producing several converted waves. A portion of the seismic energy reaches the surface of the earth, and is recorded by the seismometers. Since the radiated energy spans a wide range of frequencies, it is desirable to sample an earthquake over as wide a bandwidth as possible, for a better understanding of the earthquake source and the medium through which the waves propagate.

Over the past decade, more than 100 broadband seismometers have been operational worldwide, belonging mostly to networks like IRIS, GEOSCOPE and CDSN. The high quality digital data that is being provided by these broadband instruments now form a fairly extensive data set for modelling the seismic sources and the fine structure of the medium. Spectral analysis of earthquake records has evolved as a powerful tool for analysis of digital data, especially for deciphering the source characteristics.

Spectral analysis using the Fourier transform has been the most powerful and standard means of decomposition of a signal into individual frequency components to obtain the energy spectrum sampled by each component. The energy spectrum thus obtained, however, does not contain information about the time of initiation of these frequencies. An estimate of the variation of the spectrum with time is desirable if one is interested in frequency dependent, time limited signals in a waveform. One way of estimating the time-varying spectrum is to compute the discrete Fourier transform of a sequence of overlapping time intervals that step through a larger time series. The spectrogram[1] utilizes this procedure and has been the most widely used tool for the analysis of time-varying spectra. The concept behind it is simple, yet powerful. In order to analyse what is happening at a particular instant of time, a small portion of the signal

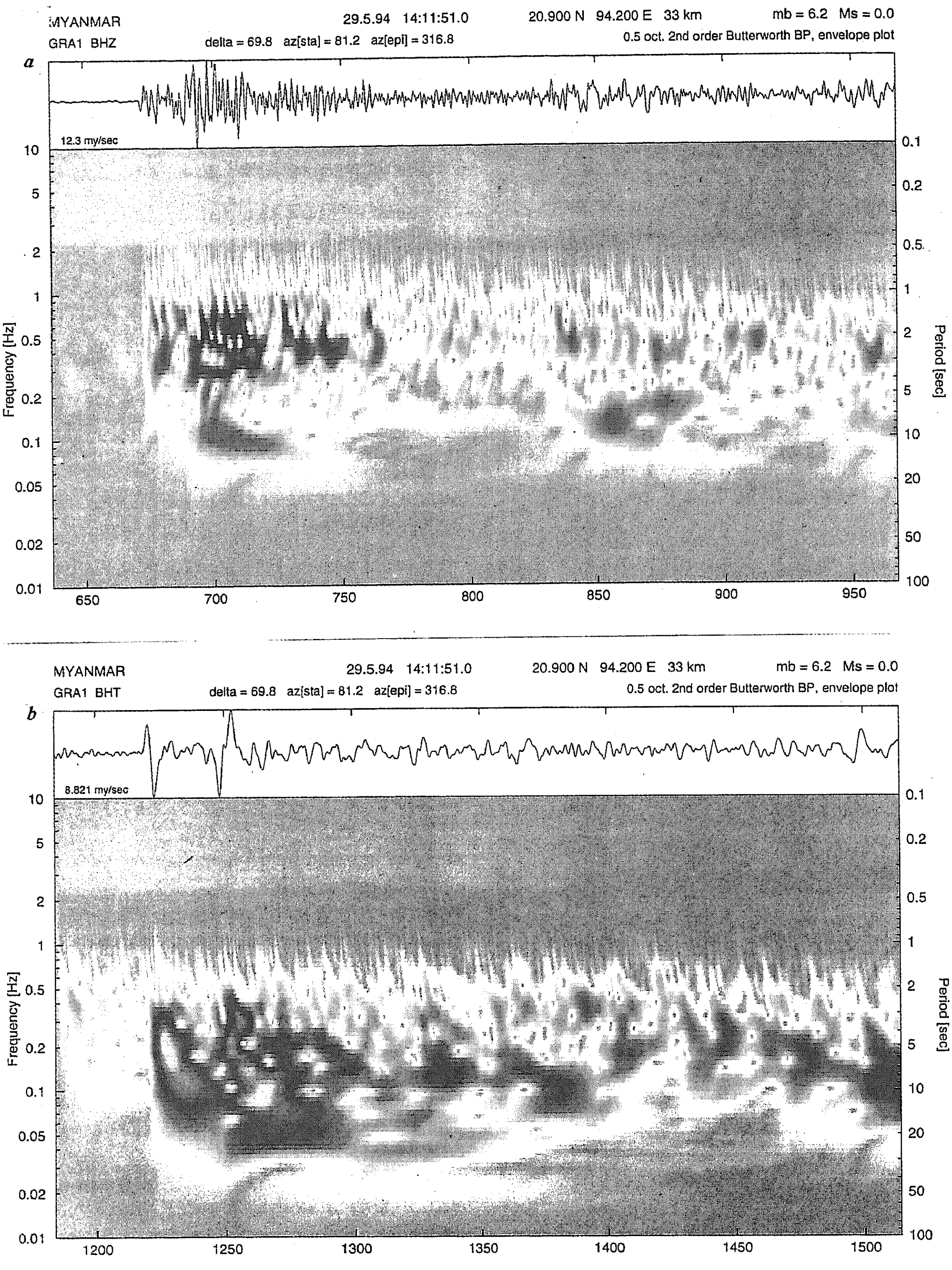

Figure 1 *a*, *b*. Spectral seismograms of the vertical and transverse components of the 29.5.94 Burmese arc earthquake recorded by a Grafenberg station. The corresponding colour code is indicated in Figure 4.

centered around that time is used to calculate its energy spectrum. The same procedure is repeated for each instant of time, using a moving window. However, the problem arises with the choice of an ideal segment duration for a moving window Fourier transformation. The window may be too short to incorporate long-period

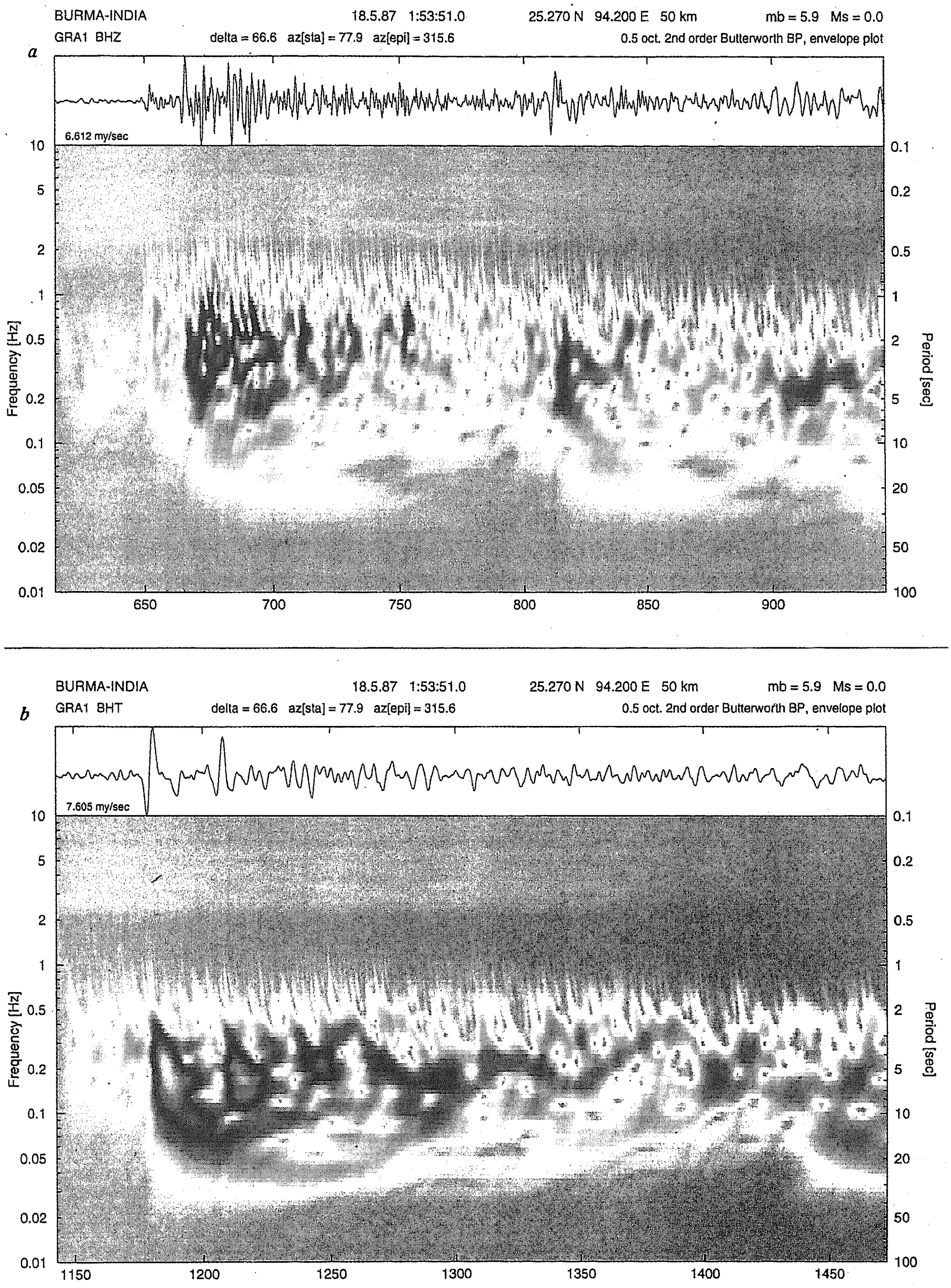

Figure 2 *a*, *b*. Spectral seismograms of the vertical and transverse components of the 18.5.87 Burmese arc earthquake recorded by a Grafenberg station.

signals adequately or may be too long to resolve changes in the behaviour of short period signals.

For an earthquake signal, the spectral seismogram overcomes this difficulty by resorting to a time–

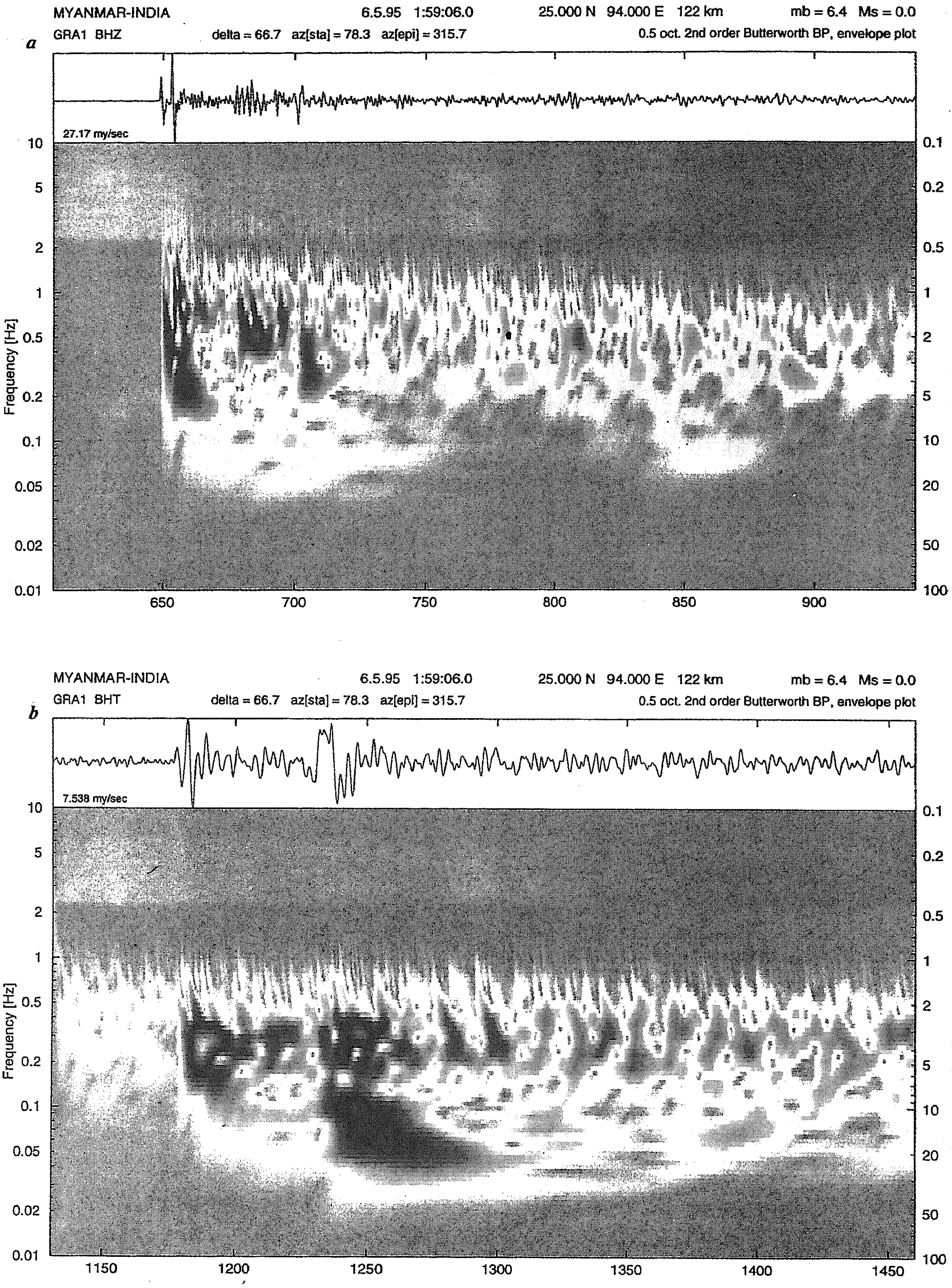

Figure 3 *a*, *b*. Spectral seismograms of the vertical and transverse components of the intermediate depth 6.5.94 Burmese arc earthquake recorded by a Grafenberg station.

frequency representation of a seismogram, using the instantaneous attributes of a signal, namely, the instantaneous amplitude or envelope and the instantaneous frequency which is the time rate of change of instantaneous phase.

A two dimensional time–frequency representation of a broad-band seismogram is called a spectral seismogram[2,3]. In this approach, a broad-band signal is passed through various narrow band filters centered at varying

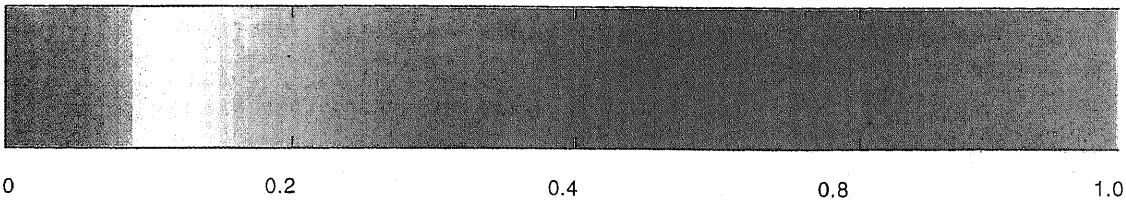

Figure 4. Colour code used to represent amplitudes in the spectral seismograms.

mid-band frequencies, to produce different bandpass seismograms corresponding to the different mid-band frequencies. The instantaneous amplitude or envelope of each of these seismograms is then computed using the concept of an analytic signal[4].

Given a real time signal $s(t)$ (a bandpass seismogram in this case), a complex analytic signal $z(t)$ may be constructed using the relation

$$z(t) = s(t) + iH[s(t)],$$

where H denotes the Hilbert transform defined as

$$H[s(t)] = \int \frac{s(t-\tau)\mathrm{d}\tau}{\pi\tau}.$$

The envelope $a(t)$ is then the amplitude of the analytic signal given by

$$a(t) = |z(t)| = \mathrm{sqrt}\ (s^2\ (t) + H^2\ [s(t)]).$$

A plot of the envelopes of all the bandpass seismograms with respect to the corresponding mid-band frequencies represents the frequency–time information of a seismogram. For the convenience of representation, each amplitude is assigned a different colour/intensity, chosen from a pre-defined smoothly varying colour/grey scale.

As examples, we have generated the spectral seismograms of several Indian earthquakes, using the 3-component broad-band data from the GRA1 station of the Grafenberg array in Germany. Though it was ideally desirable to generate spectral seismograms for the entire lengths of the seismograms, depicting the energy distribution of all the seismic phases starting from P up to the surface waves, it was found impractical to present them in a convenient size. As a reasonable compromise, we decided to choose a 5-minute duration of the P and S wave portion of seismograms after the respective theoretical arrival times, corresponding to the IASPEI 91 travel time tables.

Following the procedure described above, each seismogram was band pass filtered using a second order butterworth filter of half octave width. The mid-band frequencies were chosen so as to divide the frequency range spanned by the broad-band seismometer (0.01 Hz to 10 Hz), into 100 equal parts on a logarithmic scale. While the vertical component of the seismogram was utilized to study the frequency–time character of the P phases, the S phases were studied by computing the radial and transverse components.

In the present study, we have chosen three recent earthquakes which have occurred in the Burmese arc region, where the presence of a subducted Indian lithospheric slab is established down to a depth of about 180 km. The spectral seismograms corresponding to these earthquakes are presented in Figures 1, 2 and 3. In these figures, *a* and *b* represent the vertical component corresponding to the P wave portion and transverse component corresponding to the S wave portion respectively of the broadband signal. The spectral seismogram of the radial component is not included for convenience of representation. It may be noted that the seismic trace, along with the event information, delta and azimuth with respect to the recording station and the filter parameters used to generate the envelope plot of hundred pass band seismograms are included at the top of each spectral seismogram. The X-axis of the spectral seismogram represents the travel time, Y-axis the frequency/time period. The colour code for the amplitudes is indicated in Figure 4.

It can be observed from Figures 1 *a* and 2 *a* that there is a striking similarity in the spectral content of the P wave portions of the seismograms, in the sense that the triplets of the high amplitude around the arrival time of the PcP phase are clearly comparable. It is interesting to note from the CMT solutions that both these earthquakes are of normal type, with a small strike slip component. The shear wave part of the spectral seismograms also appears similar (Figures 1 *b* and 2 *b*). On the other hand, the spectral seismogram of the deeper earthquake (Figures 3 *a* and *b*) appears markedly different, with the P phases (surprisingly) being richer in high frequencies and the S phases richer in low frequencies, compared to the shallow focus earthquakes. This earthquake has a thrust type of mechanism. It also appears that this earthquake is a double event, as is clear on the P-wave portion of Figure 3 *a*.

It is interesting to notice concentrations of energies not coinciding with any of the 'expected' phases, corresponding perhaps to reflections from intermediate boundaries, or scattered energy. However, all the expected phases show up prominently on the spectral seismograms, making their identification easier.

Although, at present, the concept of a spectral seismogram is new and its potential yet to be utilized, it is likely to turn out as a powerful tool for routine analysis of seismograms in the near future, since it allows a detailed and quick analysis of the spectral character and time-varying behaviour of an earthquake. This tool can eventually be used to reconstruct the source time history. Also, it may find application in studies of surface wave dispersion, identification of relatively unknown phases and establishing similarities in the spectral character of earthquakes from a similar tectonic setup.

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An *Aquilapollenites*-associated palynoflora from Mohgaonkalan and its implication for age and stratigraphic correlation of Deccan Intertrappean beds

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Palynology of the intertrappeans of Mohgaonkalan of Chhindwara District, M.P. has been dealt for the first time. The palynoflorule recovered from the black shale is conspicuous due to the presence of *Aquilapollenites bengalensis*, *Azolla cretacea*, *Gabonisporis vigourouxii*, *Spinizonocolpites echinatus* and *Ariadnaesporites* sp. and is very much comparable with the palynoassemblages of Padwar–Ranipur intertrappean beds of Jabalpur. The palynological data are of significance as they now offer a fine resolution of age of Late Maastrichtian contrary to previous assignment of Early Tertiary on the basis of megafloristic evidences. Further, the association of *Aquilapollenites* palynoflora with the dinosaur remains at Mohgaonkalan has been found to be useful for correlation and tagging with the radiometric dates known from various localities of the Deccan basaltic province in Peninsular India. Considering the available palynological data and the dinosaur remains, the intertrappean beds at Mohgaonkalan are also very important for delineating Cretaceous–Tertiary Boundary (KTB) in Deccan Trap Volcanic Episode. In light of new palynological findings, a reappraisal of the Deccan Intertrappean flora and its biostratigraphic implication is needed to critically evaluate the influence of Deccan volcanic activity on the continental terminal Cretaceous biotas.

INTERTRAPPEAN palynofossils are very poorly documented as compared to plant mega fossils due to poor recovery and preservation of them in the trap-associated sediments. However, in recent years, discovery of more intertrappean horizons bearing carbonaceous facies has proved to be rewarding as the black shales in them are found to be rich in palynological contents[1,2]. While carrying out field work in and around Mohgaonkalan, we came across an unlined well that penetrated through a sequence of black shale-associated intertrappeans and this paper is based on the palynological contents yielded from it. In fact, palynoflora from this area have a significance for a fine resolution of age of the intertrappean beds of Mohgaonkalan in the Chhindwara District of Madhya Pradesh. Moreover, the new palynological data have been found to be useful for stratigraphic correlation of other palynological assemblages and the associated dinosaur-bearing intertrappean beds of the Deccan basalt province of India.

The Mohgaonkalan intertrappean sequence is exposed in an unlined water well situated about 0.5 km west of the village very close to the well-known fossil locality from where a large number of plant megafossils have been documented. The well can be easily located as local people fetch water from it for drinking and irrigation purposes. The intertrappean bed is less than 1 m thick and comprises black shale, greenish shale and light yellowish hard chert. The exact location and details of the intertrappean succession were dealt with recently where in the egg shells of dinosaurian affinities and other faunal elements have been described along with a mention of palynoassemblage[3]. The samples investigated for the present study were collected from material dug out of the water well and in fact, only the black shales have proved to be rich in palynological contents.

The palynological assemblage recovered from the black shales of Mohgaonkalan consists of *Azolla cretacea*, *Aquilapollenites bengalensis*, *Gabonisporis vigourouxii*, *Spinizonocolpites echinatus*, *Triporoletes reticulatus*, *Proxapertites operculatus*, *Tricoplites* sp., *Cyathidites minor*, *Todsporties* sp., *Ariadnaesporites* sp., *Ephedripites* sp., *Foveosporites* sp. and *Osmundacidites* sp., *Lycopodiumsporites* sp., *Alsophyllidites* sp. The palynofloral assemblage is dominated by *A. creatacea*, *G. vigourouxii* and other laevigate

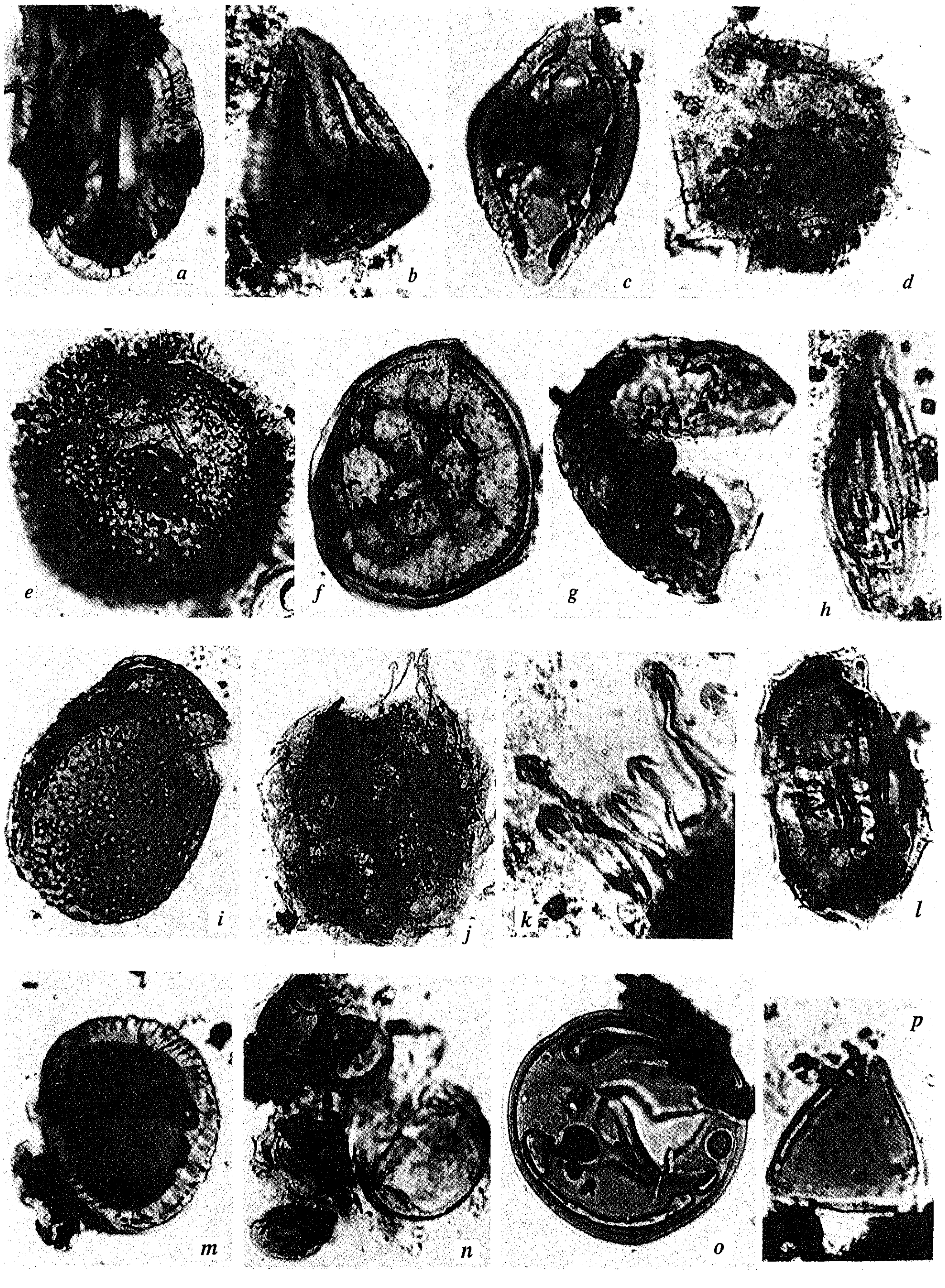

Figure 1 *a–p*. *a–c*, *Aquilapollenites bengalensis* Baksi and Deb; *d*, *Spinizonocolpites echinatus* Muller; *e*, *Gabonisporis vigourouxii* Boltenhagen; *f*, *Triporoletes reticulatus* Pocock; *g*, *Lycopodiumsporites* sp., *h*, *Ephedripites* sp., *i*, *Foveosporites* sp.; *j*, Microspore massulae of *Azolla cretacea* Stanley; *k*, Glochidia of *Azolla cretacea* Stanley ca × 1000; *l*, *Spinizonocolpites* sp. with short spines; *m*, *Aquilapollenites bengalensis* showing exine ornamentation; *n*, *Ariadnaesporites* sp. showing both micro and megaspores; *o*, *Todisporites* sp.; *p*, *Tricolpites* sp. (All photomicrographs are enlarged ca × 600, unless otherwise mentioned.)

pteridophytic spores (Figure 1 *a–p*). The detailed qualitative and quantitative aspects of the palynofloral assemblage will be dealt with separately elsewhere.

The Mohgaonkalan palynoflora is very much comparable with that of Bengal Basin, Cauvery Basin, Narasapur – well no. 1, Padwar–Ranipur and Naskal Section B assemblages[4–9] as they have common palynotaxa, viz., *A. bengalensis*, *T. reticulatus*, *Tricolpites* sp., *Ephedripites* sp., *S. echinatus*, *G. vigourouxii*, *Ariadnaesporites* sp. and *A. cretacea*. The palynological data confirm a Late Maastrichtian age for the Mohgaonkalan intertrappeans, since most of these forms are documented from well-dated strata having good palaeontological, stratigraphical and geophysical controls[8-13]. Thus, the new data have been found to be useful to resolve the discrepancy in the ages of the intertrappean beds of Mohgaonkalan. In view of the above, a reappraisal of plant megafossil records is needed to ascertain the biostratigraphic and palaeobiogeographic implications of the Nagpur–Chhindwara and Mandla assemblages[14] and other contemporaneous biotas associated with Deccan volcanic sedimentary sequences in Peninsular India. Further, the *Aquilapollenites*-associated palynoflora of Mohgaonkalan has an important bearing on the palaeoposition of India as it has Laurasian affinities while enjoying an extensive distribution in central and the eastern marginal basins of Bengal, Godavari and Cauvery in India. This paleoposition may have also allowed a free dispersal and migration of taxa between India and Eurasia through dispersal routes and terrestrial connection during the Late Cretaceous as rich assemblages of *Aquilapollenites* are distributed in northeastern China[15]. Perhaps palaeobiogeographically, India might have served as a meeting ground for both the southern and northern floral and faunal migrants during the Latest Cretaceous prior to initiation of volcanic activity.

Presence of *Aquilapollenites* in the intertrappeans has also stratigraphic implications as it is not recorded so far either in the pre-Maastrichtian sediments or in the Tertiary palynoflora of India. The global records indicate that the *Aquilapollenites* group arose in the latest Turonian and reached the peak of its abundance in the Late Maastrichtian, followed by a drastic decline to extinction in the Early Eocene[16]. Therefore, its restricted distribution during the Late Maastrichtian along with the dinosaur remains is to be considered a strong point for correlation of widely-separated intertrappean beds of Peninsular India. As there is no evidence of *Aquilapollenites* pollen that persisted across the K–T boundary in India, we have to critically assess the degree of impact of volcanic activity on the contemporaneous biota. In fact, *Aquilapollenites* being a Northern Hemisphere (circum-Pacific) form enjoying cooler climate, may have been subjected to environmental stress as a result of increase in temperature and the accompanying shift in the climate as compared to the pteridophytic spore forms that persisted for very long times. It is also likely that the Peninsular India could have been the southern boundary of *Aquilapollenites* during the Late Maastrichtian. Thus, it would be worthwhile to investigate palynoflora of more intertrappean beds in order to evaluate the disappearance of such elements with reference to Deccan Volcanic Episode that initiated just before the end of Cretaceous.

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Ballistic Missile Proliferation: The Politics and Technics. Aaron Karp. Oxford University Press, Walton Street, Oxford OX2 6DP, UK. 1996. 228pp.

Since about the mid-seventies, numerous articles and books have been appearing on India's long journey (and those of the few other nations with similar geo-strategic placings) on three critical technologies: nuclear, space and defence, including missiles. This book is one such.

Karp's 'acknowledgements' begin: 'The origin of this book owes much to the support and encouragement of three men: Maurice Eisenstein, who as an official of the US Arms Control and Disarmament Agency in the late 1970s introduced me (and much of the US Government) to the issues of missile proliferation; Walther Stützle, SIPRI Director in 1986–91, who brought me back to the issue and urged me to write this book; and Adam Daniel Rotfeld, SIPRI Director since 1991, who made its completion possible.'

The Stockholm International Peace Research Institute (SIPRI), under whose aegis this book appears, has had a reputation for authoritative study and comment on matters military and on matters concerned with the international politics of peace and disarmament. This book reconfirms what many of us have assessed in the recent past: This reputation is undeserved and increasingly unwarranted. Let us explain why.

The merits of any book dealing with issues of military policy, armaments, peace and disarmament can be calibrated by comparing the facts 'relating to India' as we in this country know them – as 'insiders' or as knowledgeable members of the informed public – with what is stated in the book. If the match is poor in the Indian case it can be very reliably concluded that the match is also poor in the other cases discussed in the book, about which we in India do not have direct information. 'Very reliably' because the 'open literature' on the Indian programmes published by official and quasi-official sources in India in the English language is so extensive that no author has an excuse for relying, as Karp has done, on some unreliable newspaper articles and other dubious secondary sources. Says Karp: '. . . all large rocket projects require some insulation from outside interference. The most successful rocket projects have enjoyed continuous leadership from one agency and often one individual manager.' Quite so. Does Karp mention Dhawan? No. But goes on to this gem: '. . . one only has to recall Korolev's role in the Soviet Union and Trien's in China. In India, the only emerging power for which comparable information is available, a similar role has been played by Abdul Kalam but without comparable institutional support. Another reason for the weakness of India's rocketry programme was the excessive concentration of power in the hands of V. S. Arunachalam, the long-time Defence Ministry Science Adviser, at the expense of development specialists such as Abdul Kalam. Switched from project to project, Indian rocket specialists were unable fully to develop any of their major projects of the 1970s and 1980s. In July 1992 Arunachalam resigned and was replaced by Abdul Kalam, a change that promises greater insulation and probably greater accomplishments for Indian military rocketry.'

Or again: 'India's Prithvi. . . is the first entirely original ballistic missile developed by a regional power other than Israel. The missile testifies to both India's technical potential and its liabilities. It relies on foreign technologies – especially for its propulsion and guidance systems. . . Because ideal technology is not available, several key systems were improvized. Instead of a preferred INS, its guidance system relies on strap-down equipment, possibly backed by radio correction (*sic*!). Unable to control direction by steering the engine nozzles, it uses large and inefficient aerodynamic surfaces. The great size of the system. . . indicates more fundamental inefficiencies. In essence, India has re-invented the Scud.' And: 'A review of missile test flights in regional powers in the 1980s and early 1990s shows continuous problems with stage separation, problems revealed only through flight-testing. Indian rocketry has been plagued by such problems. An SLV-3 launcher lifted the country's first satellite into orbit in 1980, but all other SLV-3 flights failed because of stage separation problems. Its replacement, the ASLV, was first launched in 1988 but crashed prematurely into the Bay of Bengal when its second stage failed to ignite. The first two test flights of the Agni also fell short, the first when its second stage failed to ignite. Several modifications simplified stage separation in the next ASLVs and Agnis, but in the next Agni flight the second stage failed again, this time igniting prematurely. None of India's multi-stage rockets is immune. The first launch of the ambitious PSLV rocket on 20 September 1993 ended in failure when the third stage failed to fire properly.' The facts of what actually happened to the first PSLV test flight, which are on record in the public domain, do not seem to be of any relevance to this author. Indian programmes have been assessed, judged (and even dismissed – but never mind that) by this 'expert' in a book that has been admiringly reviewed by Indians in India who should know better. He, and other authors like him, rely on the reputations of their corporate affiliations to immunize them if the facts, on which the judgements are based, are garbled or wrong.

On an earlier occasion, a 'missile proliferation expert' writing on our missile programme for the SIPRI year book requested one of us (SC) for extended comments on his draft manuscript. These were freely given. They were simply ignored. This will increasingly happen. We have to realize that the purpose of foreign authors seeking 'authoritative comment' from Indian insiders is to get the 'real dope' but only for the internal purposes of the institutions or the government (mainly Western) backing these authors. There is not, and will never be, any intention of actually using, much less acknowledging, any reliable information.

The point that we wish to advance to readers of *Current Science* is this: This is not the first time that misinformation and techno-political googlies in respect of our critical scientific and technological programmes have appeared in print. Nor will they be the last. Each time Indian S&T programmes begin to approach the current technological frontier, these kinds of articles, books and 'prestigious lectures' (some by NRIs) will proliferate, both in print and in the electronic media. It is both pointless and counter-productive to attempt case-by-case or reactive rebuttal; for, that burdens *our* time and effort, which this breed of lazy foreign author finds easy to rely on. If we do *not* rebut, their circulated conclusion is: 'Since there was no reaction, what I have said may be deemed to be the truth/facts.'

What then should be our policy? We are convinced that the only productive policy is even greater *openness* and wide dissemination at our cost of money and time, both within the country and abroad, of all except the most sensitive *technical* information. Reliable counter-proliferation with authentic public information widely pre-disseminated is the only workable antidote to the left-arm techno-googly bowled round our wicket by bowlers home-trained on the world's most treacherous academic and quasi-academic pitches.

And don't bother with this book.

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Interface Between Chemistry and Biochemistry. P. Jollès and H. Jörnvall, eds. Birkhauser Varlag AG, Klasterberg 23, P.O. Box 133, CH-4010, Basel, Switzerland. 1995. 298 pp. Price: US $ 124.

Molecular knowledge of biological process today is heavily dependent on interface between different fields. Pierre Jollès and Hans Jörnvall have attempted to highlight the importance of the interface between chemistry and biology in addressing a variety of chemical and biological problems in the book *Interface between Chemistry and Biochemistry*.

The book has articles which cover the biochemistry and chemistry of a wide spectrum of biologically active molecules. The topics are broadly classified into five sections. They are (1) chemistry at interfaces and transport, (2) chemistry and biochemistry with emphasis on enzyme activity, (3) analysis of proteins and nucleic acids, (4) synthesis of (bio) active compounds and (5) metalloproteins. Section 1 has articles on the chemistry and biochemistry of triglyceride lipases, structures of hydrophobic surfactant-associated proteins, enzyme function in organic solvents and signal peptides which sort proteins to the endoplasmic reticulum pathway. The article on lipase structures is restricted to the structurally-related mammalian enzymes and the interface aspect does not clearly come out. The article on surfactant-associated proteins is largely confined to their structure determination. The discussion on protein sorting signals appears out of place. The only article in this section that does some justice to the title of the book is the one on enzyme function in organic solvents. The advantages associated with the use of enzymes in organic solvents, the forms of enzymes used in predominantly organic medium and some important applications are clearly presented and should be very useful to those looking for such details in this area. The article would have been much more readable with a few illustrations, especially in the section where mechanisms are discussed. The articles in section 2 do justice to what the editors set out to achieve by bringing out the book. The basic principles underlying the mechanism of action of catalytic antibodies, catalytic RNA and non-biological counterparts of enzyme catalysed reaction are clearly outlined in two articles. The third article in this section is devoted to structural characterization of antigenic peptides on the cell surface of major histocompatibility complex (MHC). The emphasis has been on analysis of subpicomolar quantities of MHC-bound peptides. In section 3, there is fairly extensive coverage of chemical techniques employed for structural analysis of proteins, peptides and photodamage of nucleic acids by UV. The articles in sections 4 and 5 provide useful insights into synthesis of glycopeptides, utility of peptides in probing neuropeptide receptor of protein–protein and protein–nucleic acid interactions and Zn biochemistry.

With a few exceptions, most of the articles convey what the editors had intended to. The book will be particularly useful to chemists who would like to venture into research problems in the area of enzymes, nucleic acids or sugars.

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Fish Nutrition in Aquaculture. Series 1. Sena S. De Silva and Trevor A. Anderson. Chapman & Hall, 2–6 Boundary Row, London SE1 8HN, UK. 1995. 319 pp. Price: US$ 37.50. (Hard-bound).

The Aquaculture Series by Chapman & Hall aims to present timely volumes reviewing important aspects of aquaculture. Title 1 concerning *Fish Nutrition in Aquaculture* is a most welcome book. Firstly, the global aquaculture industry is worth about 30 billion US dollars and is growing at the rate of about 10%. Clearly aquaculture has emerged as an important industry during the last decade and is practised in more than 150 countries in the world. Aquaculture, once considered an environmentally sound practice, is now regarded to be a potential polluter. Environmental restrictions are being increasingly imposed on aquacultural activities all over the world. Therefore it has become a necessity, especially for those in developing Asian countries to know more about it.

Secondly, feed and feeding are crucial elements in any animal husbandry; regarded as the highest recurrent cost in aquaculture operation, the feed cost ranges from 30 to 60%, depending on the species, culture system and intensity of operation. Fish are known to draw metabolic energy mostly from protein and lipid, rather than carbohydrate and lipid; not surprisingly the optimum protein : energy ratio is around 1 : 2 for fish but it is 1 : 10 for ruminants. Therefore the optimal dietary protein requirement for fish is 0.5 to 3 times higher than that required by farm animals. Another aspect to be recognized is that the number of aquatic species, for which culture methods are developed to date is 195, which includes 92 species of finfish. Thus a large number of fish species are being cultured and their anatomy, physiology and feeding behaviour differ enormously. Therefore the problems associated and encountered in feeds and feeding aquatic species differ vastly from those in terrestrial animal husbandry.

Thirdly, Asia is considered as the cradle for aquaculture. Asian countries contribute approximately 85% of the total production. Admirably the authors have chosen to make a concerted effort to draw appropriate and adequate examples from many Asian farms. The book is much benefitted by the senior author who, through his frequent visits to research institutions and farms in many Asian countries, has gained first-hand information relevant to the topic and has chosen to incorporate it in this book.

The contents of the book are lucidily presented in 13 chapters; essentially these chapters may be considered under 2 sections. The first one consists of the lengthy chapters 2 and 3 on energetics and intermediary metabolism. The second one includes chapters 4 to 13 and is followed by a carelessly prepared 'References' and a useful 'Index'. The first section provides the base and the required continuity for the second. Yet most of it is a repetition of what is presented in most standard biochemistry and physiology books. In fact, Jobling (1994) has presented these basics more succinctly in his *Fish Bioenergetics*, a book in the Chapman & Hall's Fish and Fishery Sciences series; his presentation on the entry points of different amino acids and fatty acids into the citric acid cycle of fish for ATP production should have found a place in De Silva and Anderson (1995), as it does not usually find a place in most general and comparative physiology books. It is not clear why De Silva and Anderson (1995) have not even cited Jobling (1994). It is equally not clear why the editors of this book series have also chosen not to bridge Jobling with De Silva and Anderson.

It was a very useful and pleasant experience for me to go through the second section related to feeds and feeding. Little consideration has been given previously to these aspects, although this is very much required for successful practice of aquaculture. The authors have endeavoured to focus every deserving research contribution, which will reduce the dietary cost. For instance, the projection of the expected normal growth obtained by altering high protein with low protein diet in tilapias and carps may be mentioned; however, it is desirable such experiments be continued with other omnivorous fish like mullets and milkfish or carnivorous fish like murrels and eels.

The reviewer is confident that the authors will have occasion to reprint and re-edit the book. Therefore, he has chosen to point out the following mistakes. Examples for avoidable spelling mistakes; p.144 – velliger; p. 154 – Adapted from .. ; p. 177 – a formulated feed meet; an example for a howler: p.144 – nauplii of rotifers. In figures 2.6, 2.7 and 4.7, explanation is missing for Y axis; for figures 4.2, 4.7, 4.9 and 5.1, unit is not given for the explanation on Y axis. Often the authors have suggested to refer to more details given elsewhere in another chapter; one is disappointed when such an explanation is missing, e.g. on p. 262, the authors have stated that the 'notion that fish require a higher level of protein in their diet than farm animals such as poultry is being refuted' and have indicated that more details are given in Chapter 3; one is disappointed not to note relevant facts and figures to refute the notion.

Despite these avoidable mistakes and errors, the authors must be congratulated for having contributed a commendable book, which is recommended for use in libraries of research institutions, aquaculture farms and feed industries, especially those in tropical developing countries.

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CENTRE FOR CELLULAR AND MOLECULAR BIOLOGY

(Council of Scientific & Industrial Research)
Hyderabad 500 007

(A National Centre of the CSIR for Basic Research in Multi-Disciplinary Areas of Modern Biology)

Advertisement No. 3/97

JUNIOR RESEARCH FELLOWSHIPS AND POST-DOCTORAL FELLOWSHIPS

JUNIOR RESEARCH FELLOWSHIP

Director, Centre for Cellular and Molecular Biology (CCMB), a constituent of Council of Scientific & Industrial Research (CSIR), New Delhi invites applications from candidates having consistently good academic record and a strong motivation to pursue research in modern biology for Junior Research Fellowship positions. Some of the broad areas of research in which the candidates can undergo Ph D training are: (i) Biochemistry and Biophysics, (ii) Molecular Biology, (iii) Cell Biology, (iv) Genetics and Evolution, (v) Biomedicine and Biotechnology.

The applicant should have an M Sc degree in any area of biological sciences or in an area of physical sciences such as physics or chemistry and a consistent first class academic record (should have obtained 60% or more marks in each of the major public examinations beginning from the 10th standard or have successfully qualified in the CSIR/UGC joint entrance examination for research fellows) for the award of Junior Research Fellowships. A strong background in physical sciences would be an asset for those who wish to apply. **Those who are in the M Sc IVth semester are also eligible to apply. Candidates with an MBBS degree and a good academic record may also apply.**

The upper age limit is 28 years as on 10.5.1997 which is relaxable by 3 years for applicants with relevant research training/teaching experience and by 5 years for women, SC/ST and physically handicapped applicants.

All selected applicants will have to undergo a written test followed by oral interview tentatively scheduled for 21 and 22 June, 1997.

For candidates who have not passed the CSIR/UGC Research Fellowship examination, selection against the advertised position will be provisional and the regular award of fellowship is subject to the candidates passing the CSIR/UGC Research Fellowship examination.

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A stipend of Rs 2,500/- p.m. plus HRA as admissible under Rules will be paid (in case of non-allotment of residential hostel accommodation).

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Applications

Completed applications in the prescribed format as published in this advertisement for Junior Research Fellowships/Post-Doctoral Fellowships should reach the Senior Controller of Administration, Centre for Cellular and Molecular Biology, Uppal Road, Hyderabad 500 007, India, by 10 May 1997.

Mere fulfilling of the minimum requirements stipulated in the advertisement would not automatically entitle a person to be called for the interview. The decision of the Centre in regard to the candidates to be called for interview shall be final. Canvassing in any form and/or bringing in any influence, political or otherwise, will be treated as a disqualification.

Sr. Controller of Administration

APPLICATION FORM FOR JUNIOR RESEARCH FELLOWSHIPS AND POST-DOCTORAL FELLOWSHIP

Please specify whether applying for Junior Research Fellow/Post Doctoral Fellow

Affix your
photograph

1. Full name of the candidate ____________________
   (in block letters)
2. Address: a) Correspondence ____________________ b) Permanent ____________________
3. Date of birth: ____________ 4. Place of birth: ____________
5. Nationality: ____________
6. Whether belong to SC/ST/OBC/General: (please specify)
7. *Have you applied earlier for research fellowship in CCMB? Yes/No
8. *Have you qualified in the CSIR–UGC joint entrance examination (NET)? (enclose certificate in case your response is 'yes') Yes/No
9. Academic record including any technical qualifications commencing from 10th class, Intermediate, B Sc and M Sc and Ph D

Name of the Examination/Degree with the name of Inst./University	Year of passing	Class or Division	% of marks obtained	Subjects (underline the main)	Scholarships/ Awards
Class X					
Class XII (Inter)					
B Sc					
M Sc					
M Phil (if applicable)					
**Ph D					

10. Have you published any scientific papers/ technical reports? If so, please give details (attach a list) Yes/No
11. Other extracurricular interests:
12. Please write a short note (about 200 words) about your interest in taking up a research career particularly in an area of molecular biology (please attach separate sheet)

*Only for JRF applicants.

**Please mention title of thesis, name of the supervisor and university and a list of 3 referees (for PDFs only)

Date:

Signature of the candidate

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH (CSIR), NEW DELHI, INDIA

ADVERTISEMENT NO. 3/97

POSITION: DIRECTOR, NATIONAL BOTANICAL RESEARCH INSTITUTE (NBRI), LUCKNOW

CSIR, the premier agency established by the Govt. of India to undertake scientific and industrial research in the country, is looking for a suitable scientist/technologist for the post of Director for its National Botanical Research Institute (NBRI), Lucknow. NBRI is devoted to carry out R&D on conservation, improvement and exploitation of economic plants, including ornamentals; to carry out basic and applied botanical, horticultural and related phytochemical research on plants and plant products and to develop production technologies for new plant sources of commercial importance. The major R&D programmes of the Institute relate to plant biotechnology, floriculture, tree-biology, plant wealth utilization, environmental sciences and taxonomy and ethnobotany. The major thrust area programmes of the Institute are directed towards: (i) basic and applied research in plant molecular biology, biochemistry and stress physiology; (ii) standardization of agrotechniques and package of practices for ornamental plants for nursery and cut-flower trade and development of new cultivars; and (iii) development of production and nursery technology for woody biomass and energy plantation on sub-standard soils. The Institute offers consultancy services to industry/government organizations in garden layout, landscaping and other related areas.

The Institute has an annual budget of about Rs 800 lakhs and S&T staff of approximately 600 of which about 115 are scientists.

The post carries the pay scale of Rs 5900–200–7300 plus allowance as admissible to Council officials. The appointment to the post will be on contract for a period of six years or superannuation, whichever is earlier.

For further details, please contact the Director-General, CSIR, Rafi Marg, New Delhi 110 001, India. (Fax: 3710618, Telex: 031–65202 CSIR IN, 031–66147 CSIRIN, Gram: CONSEARCH, New Delhi).

Interested candidates may send their complete biodata by 30 May 1997 to the Director-General, CSIR, Rafi Marg, New Delhi 110 001.

COUNCIL OF SCIENTIFIC AND INDUSTRIAL RESEARCH (CSIR), NEW DELHI, INDIA

ADVERTISEMENT NO. 4/97

POSITION: DIRECTOR, INSTITUTE OF MICROBIAL TECHNOLOGY (IMTECH), CHANDIGARH

CSIR, the premier agency established by the Govt. of India to undertake scientific and industrial research in the country, is looking for suitable scientist/technologist for the post of Director for its Institute of Microbial Technology (IMTECH), Chandigarh. IMTECH is devoted to provide an integrated research, development and design base for microbial technology; to undertake basic and applied research and development programmes in established and newly emerging areas of biotechnology including genetic engineering; to develop and maintain gene pool resources and genetic stocks of microbial cultures and other cell lines; to impart training in microbiology, microbial technology and biochemical engineering to research workers and technologists; to establish and maintain effective linkages with industry and educational institutions, develop capabilities for producing design and engineering packages for industrial plants and to undertake contract work on research and development in the areas of biotechnology. The institute carries out application-oriented R&D in the frontier areas of biotechnology with the short-term objective of capability generation and a long-term perspective of generating newer leads in the critical areas of its working. Most of the research programmes deal with the appllication of newer tools of molecular biology and genetic engineering to develop new technologies or improve upon the existing ones, vital to our national needs. The Institute is currently carrying out R&D programmes in the following areas: molecular biology and microbial genetics, animal cell/tissue culture, protein engineering and fermentation technology. The Institute undertakes contract work for development of microbial processes for useful products.

The Institute has an annual budget of about Rs 600 lakhs and S&T staff of approximately 140 out of which about 40 are scientists.

The post carries the pay scale of Rs 5900–200–7300 plus allowance as admissible to Council employees. The appointment to the post will be on contract for a period of six years or superannuation, whichever is earlier.

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Gamma-ray diffraction

X-ray diffraction, discovered in 1912 paved the way for unraveling structure of solids in terms of location of atoms within bulk crystal structure and thus the subject of crystallography became one of the most important activities of solid state physics research. With the availability of thermal neutron beams at nuclear research reactors in the late forties, the complimentary technique of neutron diffraction got established; this technique, in addition to providing complimentary information to that from X-ray diffraction, led to unique results concerning magnetic structure of crystals. The last fifty years have seen emergence of phenomenal understanding of structure of a variety of complex materials thanks to these two techniques. Yet another diffraction technique which has a lot of promise is the gamma-ray diffraction; this technique has been used since the seventies to investigate crystal physics 'where the other techniques were not so effective'.

D. B. Sirdeshmukh has discussed the nature of gamma-ray diffraction technique and its several applications in the review article 'Gamma-ray diffraction – A powerful tool in crystal physics' (**page 631**). Monochromatic gamma-rays (of wavelength nearly 0.02 to 0.06 Å) from fairly long lived radioactive sources have been used in the gamma-ray diffractometers. The shorter wavelength of gamma-rays compared to that of X-rays provides a number of advantages in diffraction studies. After describing these aspects, Sirdeshmukh has dealt with a variety of applications with suitable examples related to the study of: structure factors, precision measurements of structural parameter changes due to phenomena such as magenetostriction, mosaicity, magnetic domain structure and rate of crystal growth.

Research based on gamma-ray diffraction may be said to be in its infancy as only a few advanced laboratories, principally in the West, have set up facilities for gamma-ray diffraction. Backed by the vast experience that this country enjoys in crystallography, it is well within our reach to undertake investigations in materials science via gamma-ray diffraction. A new thrust is needed to initiate techniques like neutral atom scattering, low temperature laser trapped atom spectroscopy or gamma-ray diffraction, if we do not wish to miss the current trends in Materials Research.

K. R. Rao

Pollution and human reproduction

Reproduction and adaptation are the two most critical attributes for survival of a living species. Humans have not done too badly on either score. Since the days of Malthus, the prospect of burgeoning human populations has often triggered off speculation about doomsday scenarios on an impossibly over-populated planet. Nowhere are the effects of overpopulation felt more than in the urban centres of the developing world. Bangalore, where this journal is based, is no exception. Paradoxically, in this issue (**page 621**) Mehta and Anand Kumar draw attention to the declining semen quality in Bangaloreans. This study comes in the wake of reports from around the world that fertility and reproductive health may indeed be on the decline. The declining quality of the environment has always been viewed as a possible cause; with several studies suggesting a connection between air pollutants and low sperm counts and diminished sperm viability.

The results from Bangalore show a significant fall in semen volume and sperm concentration and increased oligospermia, which apparently correlate with the levels of suspended particulate matter in the air. There has already been considerable speculation on the molecular origins of the connection between pollution and reproductive health. Synthetic chemicals which can trigger estrogen-like responses may indeed be prime suspects in causing cancer and reproductive problems. Indeed a paper published last year (Arnold *et al.*, *Science*, 1996, **274**, 1489) revealed a dramatic synergistic effect of a combination of two polychlorinated biphenyls.. These studies have been questioned more recently (*Science*, 1997, **275**, 1879), suggesting that the final verdict is still to come. Ironically, population control may in fact come about by an unanticipated environmental feedback mechanism. This is certainly a point to ponder when stuck on urban roads enveloped in clouds of dense automobile exhaust.

P. Balaram

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CURRENT SCIENCE

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CORRESPONDENCE

Development of cloning technology for humans

'Hello Dolly' by K. P. Gopinathan (*Curr. Sci.*, 1997, **72**, 362–364) presents an excellent summary of the achievements made by Wilmut's group at the Roslin Institute, in animal cloning and briefly discusses the ethical, social and philosophical issues that have been raised in extending the technology to humans. The cloning technology would certainly have immense advantages over the methods currently used in animal breeding. Artificial insemination (AI) from semen collected from identified, superior bulls is widely used for genetic improvement of cattle. AI however, limits the benefits of the desirable genes only from the male side which contributes half of the offspring's genome. Superovulation and transfer of embryos to surrogate mothers – the embryo transfer technology (ETT), enables selection of both male and female genetic contribution. Yet, the genetic potential of the offspring remains a matter of chance, dependent upon random segregation of the genes. Cloning would provide for selective multiplication of the proven, elite combination of genes by choice. The technique, as reported by Wilmut *et al.* (*Nature*, 1997, **385**, 810–813), however, needs further research inputs and refinement to realize high success rate, minimize induction of genetic alterations in the process and for cost effectiveness. Therefore, animal cloning research should receive increased support. Considering the potential advantage of the technique in animal breeding, private funds will be channelled to develop commercially viable cloning technologies as has happened in plants. It is estimated that current global production of *in-vitro* raised plants is around 750 million and increasing rapidly.

As mentioned by Gopinathan, and widely reported in media, extending of the cloning technology to humans has received a negative response in the US, France, Germany, Japan and from Vatican, which is not unusual for any new technology dealing with life processes, especially the human life. Therefore, ethical, social and philosophical questions need to be examined by the biologists, social scientists, decision makers and public after careful considerations of the facts. The new technologies, especially the biological one, are often misconceived by the people. The general misconception about cloning is that the clones would be the 'photostat copy' of the donor. This is far from truth. The phenotype – size, shape, appearance, behaviour, ability to acquire skills, etc. – is dependent on the genotype (the genetic content), environment and interaction of the genotype and environment during all developmental phases. The clone will have its origin from a single cell, modified to develop and grow like an embryo, and implanted in a surrogate mother. It would undergo development in the womb, just like the embryo following normal reproductive process, to be delivered after the completion of the gestation period. Women will have an added advantage that they can use their own womb to raise their clone. The only difference between the clone and the naturally conceived child would be in the genetic makeup. After birth, the clone will have to pass through different development stages, learn to talk, walk, acquire language, education and other skills. Depending upon the age at which the cloning decision is made, there will be a large age difference, and hence, the generation gap, between the parent and the clone in knowledge, social and technical skills.

Who would like to have their clones? Many new reproductive technologies such as artificial insemination, *in vitro* fertilization and embryo transfer have been available for many years now. They have been largely used by people unable to have a child through normal reproductive process, and have brought immense joy, happiness and emotional satisfaction to thousands of families. One may argue about why such technologies are needed in an overcrowded world struggling to limit the population growth. They are also expensive, and only rich families can afford them which for the overall benefit of the society should go for adoption from orphanages. Motivations to have a clone could be emotional, medical, emotional-cum-medical, ego satisfaction or a demand from a section of the population to have clones of their favourite leaders, musicians, creative artists, writers, sportsman, 'gurus' and even scientists with brilliant minds like Einstein. Considering the age difference and generation gap between the parent and the clone, discussed earlier, rapid changes in people's tastes, values and fads in highly technology-driven society, it is doubtful, if ever, the clones will have the same advantages in a different period. It is unlikely that many would rush to the cloning clinics, even if they can afford the cost. Variety is the spice of life and well-being of the society depends on the available biological and cultural diversity. Therefore, cloning would neither be widely accepted nor would it be beneficial for the well-being of *Homo sapiens*. At the same time, it is likely that the people who resort to *in vitro* fertilization from the unknown donor sperms, obtained from the sperm bank, may prefer to have a clone of one of the parents, instead of using donor sperm. When both the parents are unable to produce children due to various reasons, cloning perhaps, would be the chosen alternative.

With the growing population, one child

per family is likely to be the norm in future, and couples for emotional security may like to have cells of their child preserved in liquid nitrogen, as a 'backup' to be used for cloning in the event of untimely death. If the child suffers from leukaemia and needs regular bone marrow transplant, the best source would be the clone, also for organ transplant. Parents having a child with genetic disorder such as Duchenne muscular dystrophy (DMD) would be extremely happy to have a clone of their child in which the genetic disorder was corrected before embryo implantation. This may be possible one day as selective implantation is possible at present. Only the families having a child with such genetic disorder can appreciate the joy of having a 'normal' version of their loved one. Similarly, it may be possible to obtain a virus-free clone of a child accidentally infected with AIDS virus. These, however, will raise perplexing moral and emotional issues.

Misuse of the technology to produce one or more clones of a dictator, potentate or CEO of a large corporation certainly cannot be ruled out if the technology is available. However, this possibility should be considered on the basis of the misuse of the already available reproductive technologies. How many of the dictators or the 'gurus' with unquestioned blind following have used artificial insemination to perpetuate their genes?

Ethical issues are certainly the most difficult as the moral values differ in different societies and individuals within the society. The most common objection often raised by the religious leaders is that humans should not interfere in God's creation. Such views are irrelevant, like many other stands of the religious leaders, for example, on population control in the present society. Civilization has developed by modifying nature or as the believers would say 'God's own creations', to satisfy its needs in all aspects of life. We enhance natural immunity by vaccination, get rid of infections by antibiotics, remove or replace damaged organs by surgery to prolong the life span, and at the same time use different means to control population. All these are human interferences with nature or God's creation. Without such modifications of nature and natural processes, the modern society cannot maintain its current life style. Should there be limits set on the development of technologies that may, in future, improve the quality of life? We will go only this far, but not beyond. The development of the cloning technology should not be banned just because of the fear that it may be misused by some. All technologies can be misused and that should not be the reason to prohibit their development and use. Legal means to prevent misuse should be applied. Cloning technology, as outlined earlier, may in future bring enormous emotional satisfaction to a section of the society. At the same time, as in the human genome research, certain percentage of funds should be used to understand, ethical, legal and social implications of the technology.

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Hello Dolly

Dolly's emergence (*Curr. Sci.*, 1997, **72**, 362–364) has raised too many unwanted ethical questions rather than appreciating many things that it has exposed. First, the myth of the 'one way clock' in animal cell differentiation has been debunked and this has brought cheer to those trying to control or reverse uncontrolled cell proliferation. In food and agriculture, abundance of identical animal meat may not be possible but abundance of milk is predicted. Emergence of a world free of sex and sex-related crime is bound to make it a safer place for the females. While cloning men to yield identical duplicates may have problems because of the need for and variation in surrogate mothers, the emergence of 'do-it-yourself' technology to generate identical women appears to be in the offing. In other words, cloning Hitlers, Amins or Sukh Rams may be difficult but cloning Jayalalithas, Shiela Kauls, the Bandit queens and the like, appears a strong possibility. Let us therefore not worry about the custom made soul but worry about the emergence of a society full of clones of the above types.

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Age of Principal Investigators in EMR schemes of CSIR: How do young scientists fare

Human Resource Development Group (HRDG), CSIR provides funds for extramural research (EMR) schemes in any area of Science and Technology, including engineering and medicine. For this, applications are entertained throughout the year from Universities, Post-Graduate Colleges and other Institutes of Higher Learning including non-CSIR R&D laboratories.

No age limit, upper or lower, has been fixed for the General Research Schemes. There are retired but active scientists above 65 years of age, as well as scientists below 30 years who have received CSIR grants to pursue research under this scheme.

An analysis was done as regards the distribution of age of the Principal Investigators (PIs) as on the date of sanction of their scheme. Depending on the area in which the schemes were sanctioned, the data was then grouped into three major disciplines as under:

- Biological sciences, which included plant, animal and medical sciences.
- Engineering sciences, which included all areas of engineering, chemical and material technology.
- Physical and chemical sciences, which included physics, chemical sciences, mathematical and earth sciences.

HRDG, through a well-established selection mechanism has been supporting research schemes mainly in Indian universities for long. For this purpose, presentation of the data and relevant information in a structured and scientific fashion to the concerned Research Committee were being done, and that helped in selecting the best from among the deserving applicants. This procedure not only gave the PI a fair chance to establishing his track record at an early age, but also fruitfully culminating the research output. Promising meritorious cases are given adequate chance through deferred scheme mechanism to improve and compete.

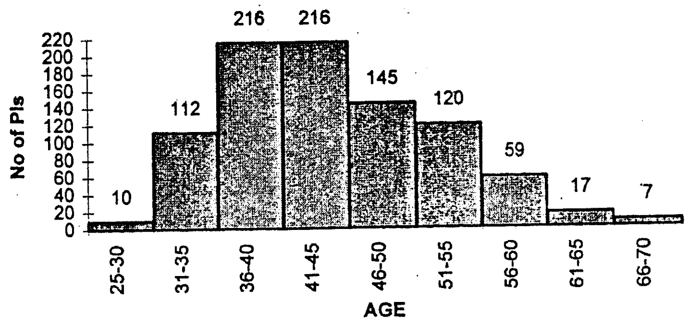
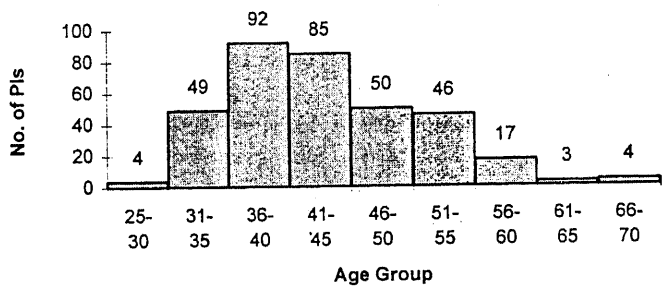
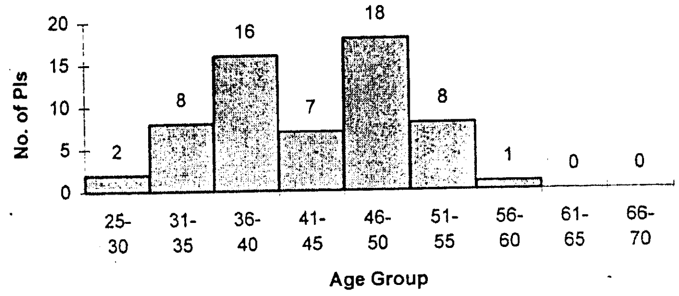


Figure 1. Distribution of age of Principal Investigators (PIs) of 902 EMR schemes (1990–97).

a. Average age for Biological Sciences



b. Average age for Engg. Sciences



c. Average age for Physical & Chemical Sciences

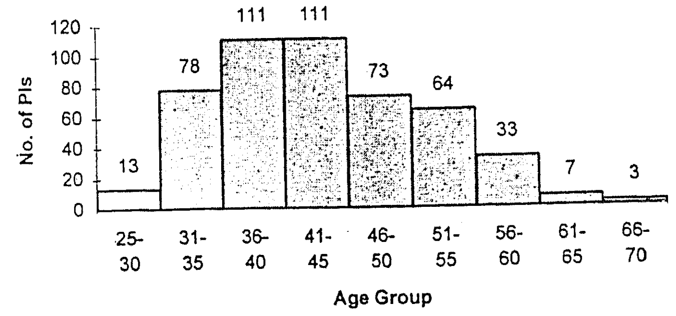


Figure 2 *a–c*. Distribution of age of Principal Investigators (PIs) in three major areas (1990–97).

A total of 902 (out of about 2500) schemes sanctioned in the last seven years (from 1990 to 1997 March) were analysed. Figure 1 shows the distribution of age of PIs in all subjects areas. The concentration is around 36 to 45 years, the peak being at 41 years. Figure 2 *a, b, c* shows the average age distribution of PIs in biological sciences, engineering sciences and physical and chemical sciences, respectively. Here also the peaks are at around 40 years of age except engineering sciences where the two peaks are identifiable, the prominent one has shifted to 46–50 age group.

In India a fresh Ph D normally gets a permanent job in the University system/ R&D institution after a few years of post-doctoral work either here or abroad and he/she gets it sometime around 30 years of age. The Shanti Swarup Bhatnagar (SSB) Award, the highest coveted award in India for recognizing research contribution, puts the upper age limit as 45 years. The average age of SSB awardees is 42 years. The SSB award is for research contributions made during the last five years preceding the year of prize. Since research work is an essential part of the activities of a faculty member/ scientist in R&D organization, it can be said that a young faculty member gets scientific independence around the age of 40 years and can manage a project independently. They are able to train younger people into R&D work through Ph D programme at this time. The age distribution concentrating around 40 years is considered on the higher side *vis-à-vis* the policy of supporting basic research in University system. Looking at the average age of SSB awardees, concentration around 35–37 years would have been ideal.

The present mechanism of peer review and screening through expert committee which has a strong scientific base is, in a way responsible for the large number of researchers coming to CSIR for getting a moderately well-funded project to establish themselves in the field of their specialization. It is felt that there is a need to improve the system (to get scientific independence) at university level so as to attract more younger researchers to take up high quality basic research.

In engineering sciences, major peak around 46–50 age group is a misfit in this model (applicable to science subjects). This may be due to several factors like:

- R&D engineers are much in demand for consultancy and other developmental work not exactly fitting into EMR type of scheme.
- Lack of adequate infra-structural facility to take up R&D work in many of the engineering degree colleges.
- Non availability of research scholars with engineering background, to work in R&D project.
- Lack of tradition to go for research work/paper publication/patent awareness in the institution.

There is an urgent need to take steps to attract younger researchers in the area of engineering sciences. Integration of appropriate academic institutions with CSIR laboratories through collaborative projects has been envisaged in CSIR 2001 vision and strategy.

ACKNOWLEDGEMENT. We thank Dr N. R. Rajagopal for his valuable suggestions.

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RESEARCH ASSOCIATE/PROJECT ASSISTANT POSITIONS

Research Associate (Ph D) and Project Assistant (M Sc) positions in a DBT-sponsored 'Facility on Transgenic Animals (Mouse)' are available at Indian Institute of Science, Bangalore. Candidates with recent Ph Ds or those who have submitted their thesis in the area of Animal Breeding and Nutrition or Embryo Biology or Veterinary Sciences (for Research Associates) and those with M Sc degree in Biological Sciences (for Project Assistants) are encouraged to contact Prof. M. R. S. Rao, Department of Biochemistry, Indian Institute of Science, Bangalore 560 012. Fax: (080) 3341683, e-mail: mrsrao@biochem.iisc.ernet.in. Please apply with a brief CV along with two letters of recommendation.

(Last date: 20 June 1997).

A new Sun from SUMER/SOHO

B. N. Dwivedi and K. Wilhelm

'The Sun will not break the rules', says Heraclitus of Ephesus; if it does, 'The Erinyes, the aids of justice, will take vengeance'. And the Sun poses a profound scientific challenge to the physicist. We do not know, for instance, why the Sun is compelled by the laws of nature to produce something as familiar as the sunspot or as unexpected as the variation in irradiance. Likewise, we do not fully understand the physical processes that heat the coronal gas to a million degree temperature or refrigerate it (e.g. prominences, filaments), that propel the solar wind and that drive the coronal mass ejections (CMEs), etc. Not only is there a whole world of scientific mystery, but its activity affects our living conditions on this planet (the terrestrial environment), from occasionally knocking out power grids to space weather and general climate. The main question is always, why does nature do it that way?

Observation from the sun-gazing spacecraft SOHO (Solar and Heliospheric Observatory) holds exciting promise to provide clues to the solar mysteries. Launched on 2 December 1995, SOHO turned an unblinking eye on the Sun on 14 February 1996, sitting at the so-called L1 Lagrangian point in space. The spacecraft carries a complement of twelve sophisticated, state-of-the-art instruments, to probe the Sun in different ways. Three helioseismic instruments in the spacecraft make a hi-fi recording of the Sun's song while the other nine instruments make a coordinated study of the outer atmosphere of the Sun and the solar wind. Like nearly all efforts to study the Sun, SOHO links events in the Sun's atmosphere and solar wind to activity beneath the Sun's visible face and the Sun's strange song.

On board the SOHO mission, the Solar Ultraviolet Measurements of Emitted Radiation (SUMER) experiment investigates the Sun in the spectral range from below 500 Å to 1610 Å at any location on the solar disk and in the low corona (0.5 $R_\odot$ above the solar limb). The SUMER spectrometer records the detailed extreme-ultraviolet (EUV) spectra. Making use of the telescope mechanism, it produces monochromatic images over the full solar disk and beyond, into the corona. The SUMER wavelength range is most suited to provide new insights into the physical processess that maintain the corona and generate the solar wind[1].

First results

The needs of astrophysics have inspired much of laboratory spectroscopy, leading to major discoveries in astrophysics including the famous Ira Bowen's identification of the nebular lines and Bengt Edlén's discovery of the million-degree-temperature corona. In a gas of very low density (e.g. coronal gas) an atom may remain in a metastable excited state for a long time (several seconds or longer) before it finally drops to a state of lower energy, emitting a photon. The understanding of this physical process played a prominent role in probing cosmic sources. Lines originating from low-lying metastable levels are called 'forbidden' lines, which are principal means of measuring plasma temperatures and densities. Spectral catalogues of different solar features have been derived from scans over the entire wavelength range of SUMER. They include emission lines and continua from neutral and ionized atoms prevalent in the temperature range $10^4 < T < 2 \times 10^6$ K. These carry the fingerprints, from the chromosphere to the corona in the broad spectral range, and provide new plasma diagnostics and dynamics of the solar atmosphere. The EUV spectra from SUMER are richer than those recorded in the past. They contain lines emitted by neutral atoms and highly-ionized atoms from a large number of elements. High-resolution EUV spectra have detected lines even from the eight-times-ionized potassium element (K IX at 636.325 Å)[2]. Making use of the observed lines from nitrogen-like ions such as Mg VI, Si VIII and S X, the density structure in the streamers has been deduced. Off-limb SUMER spectra contain very large number of unidentified forbidden lines. A great amount of effort and obviously, a virtual challenge to atomic physicists awaits the identification of these EUV lines. New dimension to the understanding of the coronal plasma is anticipated.

Recent researches show another puzzle about the Sun that element abundances in the upper solar atmosphere and photosphere are not identical. That is, in the corona, heavy elements with low-FIP (first ionization potential) (< 10 eV) appear preferentially enhanced by about a factor of four or so relative to high-FIP ones (> 10 eV). Some key issues concerning the enrichment of the upper atmosphere with low-FIP elements can be answered by SUMER. Are all low-FIP elements enriched by the same amount? Are the most abundant low-FIP elements such as Si (8.1 eV), Fe (7.9 eV) and Mg (7.6 eV), enriched to the same degree as the low-abundant elements namely, Ca (6.1 eV), Al (6.0 eV), Na (5.2 eV) and K (4.3 eV)? SUMER provides rich spectral signature of these low-abundant elements for the first time. We have recorded detailed EUV spectra for Ne VI and Mg VI lines, using the SUMER spectrometer for deducing density and low-FIP to high-FIP element variation in different solar structures. Analysis of the spectra is underway[3]. Abundance anomaly would affect a range of problems including emission measure distribution, model solar atmospheres, energy equation, spectroscopic line ratio diagnostics, radiative loss function, etc.

Among the many theoretical concepts of how the Sun heats its corona, magnetic reconnection process plays a key role. Small-scale reconnection events in the transition region have been observed previously by HRTS. SUMER with its excellent spatial, spectral and temporal capabilities, permit the investigation of such events in much more detail. For example, an explosive event was recorded by SUMER on 1 May 1996 in the O VI 1032 Å line at about 3×10^5 K. Several fascinating explosive events have been observed in different suitably selected lines by SUMER team since then. Analyses of these events hold a good promise to address the coronal heating riddle.

SUMER has already imaged various solar structures, on the disk (e.g. network, plage) and at the limb (e.g. prominences, coronal holes, streamers, plumes). This data set exhibits the power of combining high spectral resolution, multi-line (i.e. multi-temperature) spectroscopy with the best spatial resolution ever obtained on a solar free-flyer spacecraft. We present here an image of a solar prominence in

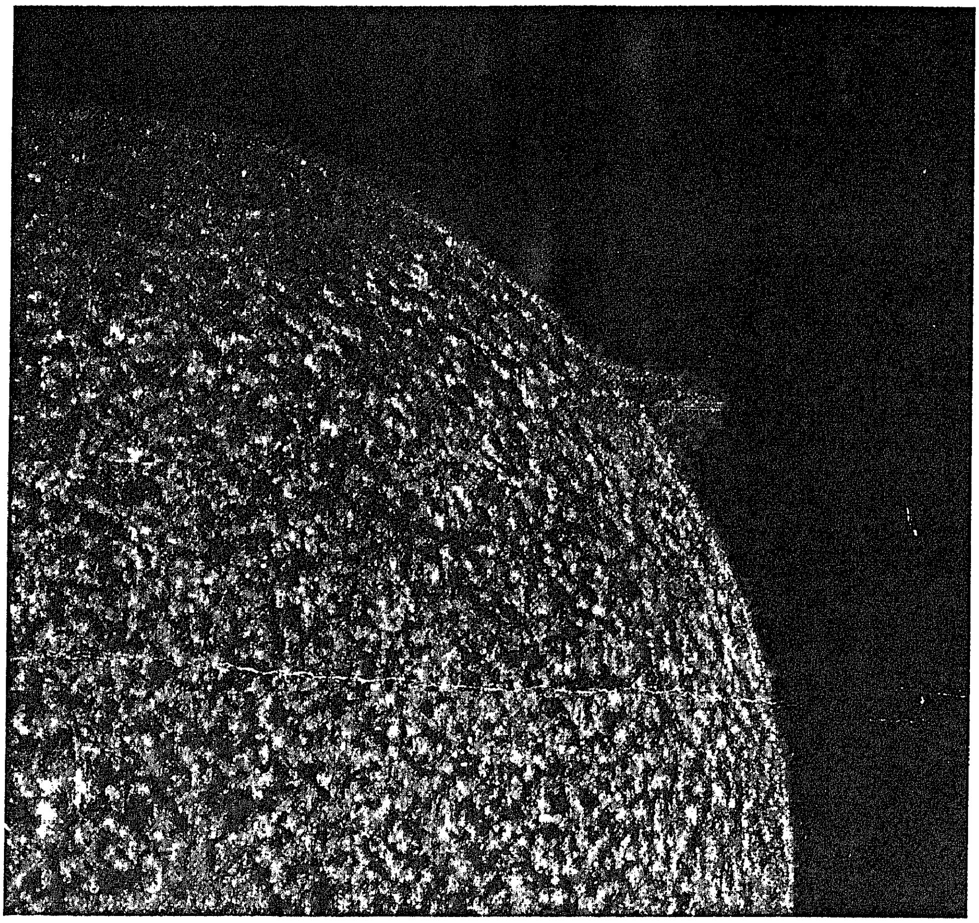

Figure 1. The Sun's limb with prominence in the emission line of neutral helium at 58.43 nm wavelength taken by SUMER on 4 March 1996.

neutral helium line at 584 Å taken by SUMER on 4 March 1996. Spectral shapes of this line in the prominence and on the disk are studied[2]. The prominence profile is about 20% narrower than that on the disk and is shifted towards blue by 40 mÅ. This corresponds to a line-of-sight velocity of 20 km/sec. The ability to measure spectral lines with such precision allows us to determine accurately temperature and turbulence from line widths, and bulk velocities from line shifts in prominences and filaments.

Visible inspection of EUV spectral signatures obtained by rastering the solar atmosphere either on the disk or off-limb, establishes the very dynamic nature of emitting source. The spectral lines get brightened or weakened from one position to the other, indicating inhomogeneous and non-isothermal solar plasma. But what amazes us most is that some strong lines disappear abruptly while other lines appear in the spectra as we raster through the atmosphere. The Sun thus sparkles like diamond in the EUV high resolution spectra, not seen before.

In conclusion, high-quality EUV spectra present an exciting promise to address more satisfactorily the riddles of coronal heating and solar wind acceleration. Identification of many intense lines (from atoms, ions and their transitions producing these lines are presently not known) in the SUMER spectra is a challenging task. These new lines, not seen before, present a new Sun with new elements, new diagnostics and probably new clues to our understanding of the solar atmosphere. 'Do you hear the chemical accents of the Sun? . . . And from all this, do you make calculations?', so says D. H. Lawrence in '*Bare Almond-Trees*'. And SUMER/SOHO from its listening post 1,500,000 kilometers out in space, records chemical accents of the Sun which present a real scientific challenge to the physics community for carrying out their calculations to unravel solar mysteries of what we call 'A new Sun from SUMER/SOHO'.

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Potassium tempers earth's core

A. V. Sankaran

The core of the earth, a region some 2900 km deep below, and commencing beyond the mantle zone, contains a third of the total mass of the planet, but forms hardly 14% of the volume. Studies of the seismic wave propagation through this core region, have revealed a solid central portion (about 5% by volume) and surrounding it, a liquid outer zone. In the early stages of our understanding of the earth's interior, scientists thought that the core may be a form of metallic silicate or even metallic hydrogen. But later experiments subjecting silicates to high pressures, disproved this view; also, theoretical calculations indicated that pressures of the order of 18 Mb, not prevalent in the core, are required to convert molecular hydrogen to metallic state, and this view was also dismissed. Based on geochemical abundances of elements in earth and the densities of the core, it has now been established that the core is essentially made up of iron. While these are the broad features of the core zone lying beyond the lower mantle, certain physical features of this region have generated considerable debate among scientists about possible presence of other chemical constituents here, besides iron. For example, seismological measurements show that the core is less dense than pure iron at the core temperature and pressure and this has given rise to speculations among earth scientists seeking to explain its implications on the chemistry of the core.

At the mantle/core transition zone, the density has been estimated to increase from 5.5 g/cc in the mantle zone to 10 g/cc in the core at a pressure of 1.35 million atmospheres. Also the mantle density at the mantle/core interface is found to be 10% higher than expected. This would suggest that the mantle material is soluble in the core and that there has been intermixing between these two adjacent regions[1]. That some lighter constituents have dissolved into the core is also favoured by the proponents of the dynamo theory[2] of earth's magnetic field/electricity production, as this model requires a fairly high electrical resistance of core materials relative to pure iron for sustaining eddy current movements required. Incorporation of U, Th and K has also been attributed to sustain the convection currents through radiogenic heating of the molten core. Considerable experimental work has been carried out since the 1960s on the solubility of elements at pressures and temperatures prevailing in the core and over the years elements such as K, Mg, C, H, S and O were proposed as the core constituents to explain its reduced density[1,3].

Under high pressures such as prevailing in the core region, chemical elements display characteristics that profoundly differ from those seen at ambient conditions. For example, elements that behave like insulators on the surface have been experimentally found to be good conductors of electricity while some others that do not react with certain group of elements are observed to combine with them, at core P and T conditions. Normally, the physical properties like electronic configuration, ionic radii and other parameters of alkali metals like K, Rb or Cs differ in comparison to those of the transition metals such as Fe, Ni or Co. These two groups do not have any chemical affinities and do not react with each other, but researchers have found changes in their size and electronic structure under high pressures and the former group of elements behaved like the latter transition metals and chemically they could combine. At Pennsylvania State University, John V. Badding along with his colleagues Laura J. Parker and Toshiyuki Atou have recently achieved one such unconventional union – that of K and Ni. They took elemental powders of K and Ni and subjected them to very high pressures of the order of 310,000 atmospheres (31 GPa) and above and temperature of about 2500 K, in a laser-heated diamond anvil[4], an apparatus wherein the sample mixture is squeezed between a pair of gem quality diamonds (Figure 1). The authors collected X-ray diffraction patterns before and after laser heating and found that 'before laser heating the diffraction pattern of the sample was characteristic of a mixture of K [in the high pressure phase K(III)] and Ni. After laser heating, the diffraction lines due to K(III) completely disappeared and new diffraction lines appeared, indicative of reaction between K and Ni to form an inter-metallic compound.' Increase of the pressure up to 37 GPa combined with laser heating made the lines sharper and more intense (Figure 2).

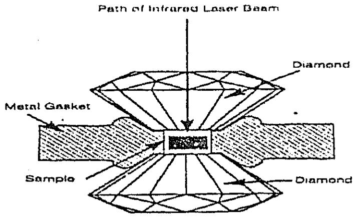

Figure 1. Diamond anvil for subjecting K and Ni to high pressures (up to 37 GPa) and temperatures (2500 K) by infrared laser from Nd–Y LiF laser.

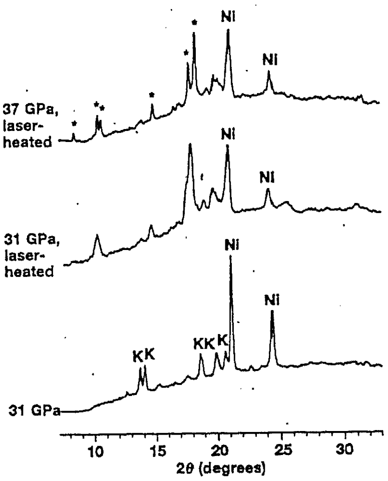

Figure 2. X-ray diffraction patterns of K–Ni mixture under pressure before and after laser heating, [after Parker *et al.*[4]].

The high pressure attained in this instrument could reduce the size of K atoms to a fifth of their ambient size and force an electronic structure analogous to the transition metals. The results have spurred Badding and co-workers to speculate that 'since charge densities of Fe and Ni are similar, despite the slightly lower electronegativity and work function of Fe', K and Fe should react under pressure prevailing in the core and the 'presence of K in the core becomes a viable hypothesis'. They feel that their results are 'important in understanding partitioning of trace elements between core and mantle, a key problem in mantle geochemistry and evolution of Earth'.

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Solar, earth and human capitals, and sustainable development

T. N. Khoshoo

The three capitals

All developmental activities are ultimately based on three types of capitals[1] which make earth habitable for human life in its present form. Broadly speaking these are solar capital, earth capital and human capital (Figure 1). The equatorial–tropical–subtropical belt receives the maximum solar energy throughout the year. This belt acts as the heat engine of the world and sets a temperature gradient from equator to poles with the associated climatic processes. Solar capital is one of the important ingredients of a deceptively simple process like photosynthesis which uses this energy into making of actual usable materials. Photosynthesis takes place in phytoplankton in sea and other water bodies and on land in plants.

The earth capital (or what may be termed as natural resources) includes resources like air, water, land, soil formation and all that is on the land and under it: e.g. forests, biodiversity, grasslands, wetlands, oceans and metallic and non-metallic minerals (including oil). Among these, wind, water, geothermal heat and biomass are potentially renewable. The earth capital includes natural processes like detoxification, dilution, decomposition and recycling of vital chemicals like carbon, oxygen, nitrogen, phosphorus, sulphur and above all water. It also includes renewable energy of sun, wind, flowing water, geothermal heat and biomass. The earth system is not static but has been changing over periods of time. It has also the capacity of self-renewal and purification. Its biodiversity has capacity for evolution and adaptation to changing climatic conditions including natural pest and disease control.

The earth capital also has life-sustaining capacity and on account of several factors has been favourable to the origin, evolution and diversification of life ever since the first self-replicating DNA molecules arose over 3.8 billion years ago. The pinnacle of evolutionary process is the origin and evolution of *Homo sapiens*. There have been many species of microorganisms, plants and animals that have come and gone, but some have stayed on the earth ever since their origin.

Although a moot point, what if from today, sunlight does not become available to earth! Much of the life as we know will come to an end because of being directly or indirectly photosynthesis-dependent. Some forms of life may spread: e.g. the biota found deep down on the sea floor or that exist in and around volcanoes on the bottom of the oceans where there is no sunlight.

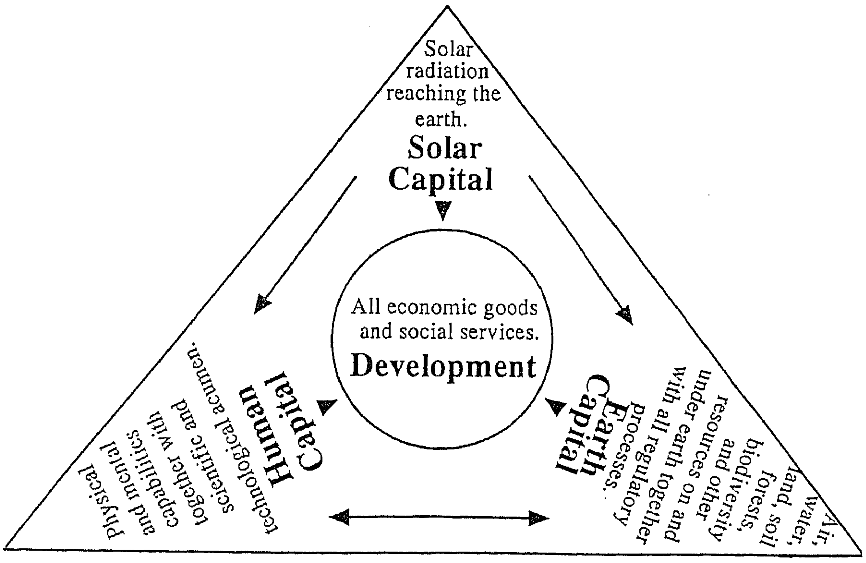
Solar radiation reaching the earth.
Solar Capital
All economic goods and social services.
Development
Human Capital
Physical and mental capabilities together with scientific and technological acumen.
Earth Capital
Air, water, land, soil forests, biodiversity, and other resources on and under earth together with all regulatory processes.

Figure 1. The three capitals for development.

The solar and the earth capitals constitute the most important components of the life-support system of the earth to be used by all species including human. The solar capital gets converted into goods and services through photosynthesis. The human capital resides in the human ingenuity, diversity, ethnicity, and diversified history, culture, religion and philosophy leading to varied technologies and socio-economic systems.

From the interaction between earth capital and human capital (technology) there emanates the manufactured capital. It includes manufactured goods using tools, machinery and equipment, and physical and mental capabilities and talent of human being. In this system technologists evolve technology, managers put it to use and look after the manufacture of goods, and workers do the actual work. The entrepreneurs invest monetary resources and then reap the profits. Thus economics is basically production, distribution and consumption of goods and services to satisfy people's wants and needs. Therefore, the three capitals together are the major source of all economic development that takes place on the earth (Figure 1). If used judiciously and with thought and care, these capitals can reform the face of earth to the good, the benefit and the well-being of not only human species but also of all creations.

The impact of human capital

The story of human species on earth begins with its ancestors like *Australopithicus afarensis* (3.36 millions years ago). After about 2 million years, arose the genus *Homo*: first it was *H. habilis*, then *H. erectus* and finally *H. sapiens* (400,000–150,000 years ago) which along with its genetic diversity also acquired physical, mental, social and cultural diversity[2,3]. *H. erectus* and particularly *H. sapiens*, colonized all the continents of the world except Antarctica.

H. sapiens (or the human being) has

been the most intelligent and a thinking animal that the earth has hosted, and has reached the present state through three types of societies as a result of three major revolutions. The first revolution was the *Stone-tool Revolution*. Here the first resource was food (plants and animals), second resource was stone which the then human being used to defend itself and also to kill its prey and do other jobs. These resources together with fire-making ability gave the then human being a distinct edge over all other animals. It not only became a thinking animal but also had an innovative mind. At this stage it was essentially a society of hunters and gatherers: men hunted and women and children gathered.

Thanks primarily to women, imperceptibly there developed an agricultural society and then followed *Agricultural Revolution*. Soon land and water became important resources for cultivation and irrigation of crops respectively. These changes accompanied the invention of wheel, plough and domestication of crops and animals. This helped in insulation of the human being against vagaries of nature by ensuring supply of food. A fallout of the agricultural societies was that human being became a 'son of the soil' (*Bhoomiputra*). These changes took place during the last 6–10,000 years.

Around 1712 AD two things happened[2]. Firstly, wood began to be replaced by coal as source of energy; and steam engine was invented. This was the beginning of the third revolution: the *Industrial Revolution*. This revolution is only about 285 years old but has been a 'mixed blessing' inasmuch as living standards of humans improved but quality of environment deteriorated increasingly. Progressively there was greater damage to earth with lowering of the quality of environment on account of overpopulation, soil erosion, loss of forests and biodiversity, pollution of air, water and land, global warming, ozone depletion, waste generation becoming a health hazard, etc. This led to environmental, developmental, economic and even political problems. At the root of this were the doings of just one species: the *Homo sapiens*. Thus from the biospheric point of view, the origin and association of human species with earth has been a 'mixed blessing'.

In this regard there are some important precedences. Some 6000 years ago, there were six flourishing contemporary civilizations which fell like house-of-cards. These were: European–Mediterranean, Babylonian, Nile Valley, Indus Valley, Huang Ho and Mayan[2]. The principal underlying reason for their fall has been disrespect for environment. Progressively the human species became the most *unnatural* species that has ever existed on the surface of earth. But the basic fact is that human being is the only species that has power of intelligence to enable to modulate environment so that it may suite her/his convenience. Thus humankind is able to evade natural selection to a large extent through the application of science and technology in which there has been tremendous growth in knowledge. Such ingenuity led to creation of artifacts. Furthermore, a human genotype which may have congenital defects in her/his organs in the body can also evade natural selection and continue to live and, what is worst, even leave progeny. All she/he needs is enough money to buy the most modern medicaments and healthy organs to replace the defective ones. One can also change an ugly face into a beautiful one.

Human being invented or discovered drugs (e.g. antibiotics in mid-1940s) to control some of the vicious diseases. This era started with the discovery of penicillin which was hailed as a major step in disease control. Initially the results were miraculous. There was a spate of new antibiotics discovered. Soon a race began between microorganisms and the discovery of increasingly more potent and new antibiotics. The race has not ended. Today we have strains of pathogenic microorganisms which are not only drug-fast but, what is worst, *feed* on antibiotics. This is most ominous: an altogether new race has begun between humans and the tiny microorganisms.

The moral is that by the use of science and technology we are trying desperately to perpetuate human genotypes which would normally have little or even no selective value in nature. In this process human being has pitched itself against the natural laws. Secondly humans have entered in a race against harmful microorganism, where the indications are that the battle is unequal and humans will remain pitched against these organisms perpetually. Their genetic system is simple and capable of countering what humans can do. In the long run, it is, therefore, an unequal battle and microorganism and even insects seem to have an edge over human beings.

Interaction between earth and human capitals

Earth has been regarded as Goddess by the Greeks (The *Gaia* concept), we in India regard her as Mother – we call her Mother Earth (*Dharti Mata*)[2]. The Gaia concept became popular thanks to Lovelock[4], an outstanding atmospheric scientist. He concluded that earth is a 'homeostatic living organism' and felt that the Gaian concept could become *a scientifically verifiable religion*. There is, however, a difference between the two concepts. Goddess is generally put on a pedestal and one bows before her and worships her; *but* with mother we have an organic connection through an invisible but indelible and permanent umbilical cord, which lasts throughout ones life; we are her children in every sense, we seek her benevolence, we depend on her and draw sustenance from her. She also provides an abode for human race and meets all the needs. In fact this is true for all the living creatures be it plants, animals or microorganisms. Ultimately, mortal remains of all organisms return to Mother Earth. There is a subtle distinction between concepts of *Gaia* and *Mother Earth*, but both are basically reverential in character.

Responsibilities of human being

With all the knowledge human being has, it is clear that so far there is no concrete evidence of life on any other planet in our solar system except the earth. Thus earth is not only unique but is also indeed a 'miracle'. The human race has a major responsibility to save this miracle in space and time because human being has also vast knowledge and power at its command. It can peer at the earth both from outer space and also while sitting on the earth itself. The changes being made by human being may be subtle or obvious, but ultimately are fouling the earth's atmosphere, hydrosphere, lithosphere and biosphere. The subtle changes in quality of air, water and water bodies, movement of glaciers, vegetal cover, forests, deserts, soil, even individual species and biogeochemical cycles together with energy flow, can now be constantly followed and measured. What is equally important is that such information can be transmitted within seconds to any part of the earth. Never before had human being acquired such a power for instant gathering of data, instant analysis, drawing strategy and conclusions and spreading the message, and also have the feedback in record time from village to the country as a whole. Such information can also be used to evolve a repair strategy and save our country and the region from ecological damage. It can also help to evolve a local to global overview and have an attitudinal change from exploiter/destroyer to saver/helper.

The repair strategy is essentially slower than power of disruption and destruction. It takes millennia to reach a stage of a climax forest but only a few hours to destroy such a handiwork of nature with myriad species that have made it their abode over millennia. *There is now a need to question the very role humans have played on the surface of this planet in changing the biosphere.* Such a change has been more for the worse than for the good of all other species.

The human being has spread to all the continents except Antarctica. It is now trying to colonize Antarctica but for a different purpose. Therefore, the effect of human presence on the Planet has made obvious and subtle changes in the life-support system. These are not only local but some changes have had global implications. The extravagant lifestyles of the industrial countries are no longer a concern of only those countries but also of the developing countries, the reason being that local changes add up to become global changes. There is only one earth and we are all interconnected, interrelated and interdependent.

The damage to earth is increasingly overshooting its repairing capacity. This is indeed a matter of deep concern for all humanity. Therefore, it is high time that the entire human race irrespective of cast, creed, or colour joins hands to repair the damage it has already done, or is in the process of doing. For this, there is an urgent need to evolve a code of conduct for human race, because otherwise our only abode will cease to support us. For instance, we may have drawn so-to-say a moratorium on the nuclear activity but the nuclear haves have not abandoned nuclear arsenals. The world is unequal, not only regarding nuclear power but also the damage inflicted to the earth on several other counts (including over-use of resources).

Today we have knowledge and power to create wealth from waste; raise forests and improve their diversity or raise plantations to meet the wood needs in a matter of decades on denuded and abandoned land; conserve species; try to improve the quality of air, water and land; try to reverse pollution; harness energy from sun, wind and water; redesign crops with the knowledge of genetics, breeding and biotechnology; use microbes to do some beneficial tasks (e.g. manufacture of insulin and other products); take to use of natural products from medicinal and aromatic plants, natural oils, gums, dyes and what not, but we cannot recreate species already lost. We have controlled population of unwanted weedy species, so we also need to control our own unwanted numbers.

Thus human race has vast and myriad powers but we need courage to restrain and use these only for the good, the benefit and the well-being of our atmosphere, hydrosphere, lithosphere and biosphere of which we are an integral part. We have to be on the side of life and the living biota and the life-support system but never ever on the side that kills the 'goose that lays the golden eggs'.

The present day crisis in environment and development is actually an outward symptom of a inner crisis in our mind and spirit about the type of society we are trying to build where human numbers are outstripping increasingly the diminishing resources of the earth, whose carrying capacity is in jeopardy, we are generating waste on an unprecedented scale, and the very security and functioning of biosphere is getting impaired. Is this the type of civilization we should build?

Furthermore, the doings of human being are such that the biosphere functioning is being impaired increasingly due to climate change, CO_2 increase, ozone depletion, etc. In every sense we are destroying and undermining our own future. Thus human race has unleashed a situation which may fast become out-of-control and human species is likely to be affected adversely.

Earth without humans

The question arises as to what happens to Mother Earth if by some chance the entire human race gets annihilated all of sudden, leaving behind all the artifacts (buildings, palaces, castles, roads, automobiles, aeroplanes, railways, industries, power plants, shopping arcades and all other infrastructure) that have resulted from human genius. Thereafter, what would be the scenario on the earth, say after 2 to 3 centuries.

All that was created by human being would have deteriorated. The buildings would have crumbled, all means of transport would have rusted, and all open spaces, roads, fields, agricultural land, parks, aerodromes, etc. would have been colonized by trees, shrubs and herbs and animals of sorts.

Most of the natural biodiversity including endangered species and forests would have flourished. However, all agribiodiversity crafted by human being would have perished. Such diversity is essentially unnatural (created to fulfil needs of the humans) and therefore bizarre and depends on its sustenance of human beings. Reciprocally human being depends on it for its own survival. For instance, the 3 to 4 feet tall wild form of *Brassica oleracea* (commonly called wild cabbage), still growing wild in European Mediterranean coast, will flourish, but the six different vegetables (e.g. cabbage, cauliflower, brussel's sprouts, kale, broccoli and kohlrabi) selected and literally crafted by human being over a period of time would have ceased to exist because these are bizarre and highly specialized with no selective value whatsoever in nature. In cabbage the whole plant has become a gigantic bud, cauliflower and brussel's sprouts are highly condensed but large and soft inflorescences of very minute sterile flowers, broccoli has large auxillary buds (mini cabbages), kohlrabi is a swollen, soft and leafy stem, and kale is indeed a very leafy vegetable (Figure 2).

Similarly, the ancestor of maize with only a few grains which are adequately protected (not naked like maize grain) and which can shatter, would flourish (Figure 3 *a*, *b*); perhaps so would modified teosinte whose grains were non-shattering but threshable (Figure 3 *c*). The cultivated maize having 'naked' grain without the hard casting of the ancestral species (Teosinte) evolved into a highly specialized type of cob specially crafted to fulfil the need for high yield. On account

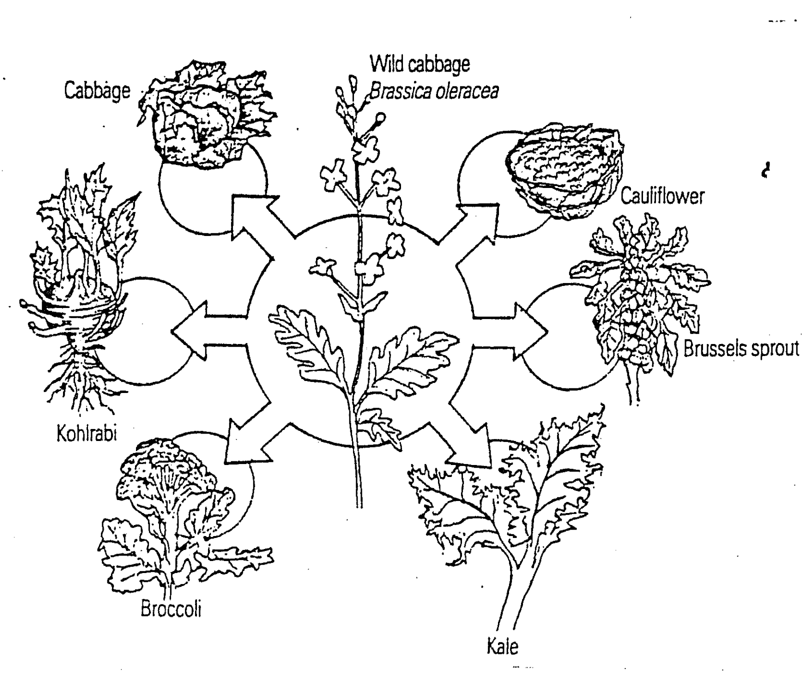

Figure 2. Wild *Brassica oleracea* and the six over-specialized cultivars developed from it by human being[5].

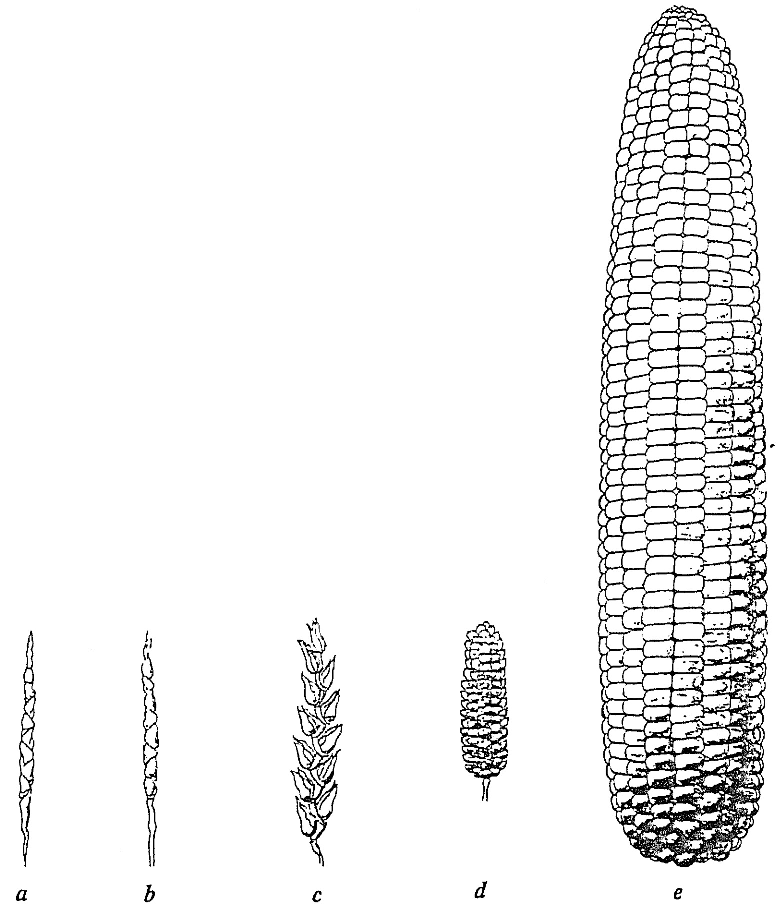

Figure 3. Stages in the evolution of cultivated maize[5].

of this the modern corn (Figure 3 *e*) would become extinct particularly because seedlings would have to penetrate the leaf-like sheaths protecting a cob. Then the seedlings would be densely clustered and compete for water, soil and nutrients and fail to reach reproductive stage[5] (Figure 3 *e*). Maize is so specialized that it would become extinct without human intervention, because it does not have any selective value in nature. The same is true of other crops and domesticated farm and non-farm animals (e.g. many breeds of dogs selected from the wild wolf, *Canis lupus*) selected by human being over the years.

Human ingenuity has through successive breeding and selectoral cycles tampered with reproductive processes of the agricultural plants and animals. The investment of their energy has been in reproduction leaving little for their defence and survival (see Figures 2 and 3). The story of agriculture has been that from hardly any worthwhile yield per unit of area and time in the wild progenitors, there has been investment to boost the yields in cultivars, be it, wheat, rice, maize, potato, pig, cattle, chicken, etc. For instance, in chicken the egg yield per hen per year has shot up from about 13 per year in the wild to over 300 in domestic breeds, making chicken merely egg-laying machines.

All the cultivars/domesticates are over-specialized and thus have become over-dependent on human being having been evolved only to fulfil food needs of humans. Therefore, these cannot face natural selection and exist without the intervention of human being. Associated with such transformation there have been skeletal and a number of other deformities which, like cultivated plants, make the domesticated chicken totally unfit to face natural selection.

The marine life in coastal/mangrove regions and in deep sea would improve in absence of humans because there would be no extraction of edible marine animals, sea weeds and corals. Furthermore, there would also be no dumping of pollutants and wastes in these habitats. In addition, the natural environment would have improved, e.g. quality of air, water and soil would have become much better. The reason being that the sources of present-day pollution would have ceased to exist. However, the host of non-biodegradable synthetic chemical compounds that are alien to biosphere, but manufactured and used by human beings, would persist, unless some microorganisms begin to feed on them and degrade the same into elemental forms.

No doubt human being in its present form has become the *most unnatural species* existing on the surface of earth. This would be increasingly so in the future. All the elements that epitomize human culture and civilization (e.g. literature, art, music and all other finer things of life) would vanish in absence of human interest and creativity.

Increasingly, one gets a distinct feeling that the earth together with most other species will fare much better without humans, because humans have created a type of civilization that is leading to the destruction of the earth. We have, therefore, to change our ways if we want to be a part of the biosphere in perpetuity. We need to change our mindset regarding unlimited growth and development, jobs, consumption patterns and the politics of domination that is behind some of the unstated objectives. Furthermore, the general feeling that sustainable development can be achieved only with better technology, laws, agreements, treaties and enforcement is true only to some extent; because ultimately it is a question of ethics and morality behind resource use. There is no alternative to this change. We have to learn new values, new imperatives and move in a determined manner towards sustainable society. There is not even one action big or small taken after 1992 (United Nations Conference on Environment and Development) towards sustainability, notwithstanding the fact there is also a Commission on Sustainable Development. It is largely a business-as-usual situation.

The fundamental point is that we have to practice ethics and morality not only *vis-à-vis* humans but also for all other creatures (plants, animals and even most microorganisms). We must respect not only human life and affirm our responsibility both to our near and dear ones but towards all life and all creatures. Over-use of materials (living and non-living), in strict sense, also amounts to violence against nature as a whole.

Homo sapiens is indeed different from all other species, because it is a thinking animal: it can *recall* its past and gain from it if it wants to. Furthermore, it can *foresee* the future. But whatever decisions emanate will have to be moral and just. Transition to sustainability must become our moral and ethical obligation to generations that will follow us. Here each one of us has a role. Each individual and her/his action is indeed critical for society, because a society is an extension of an individual. *The bottom line is that biosphere does not need us, but it is we who need biosphere for our well-being.* Therefore, there is need for serious introspection. *The 'enemy' of environment is within each one of us.* Nature is very complex, we have not understood its ways fully, we must approach it with tremendous humility, awe and respect. We must accept the fact that environment is most critical for our ecological, social, economical, ethical and even military security. These are parts of one whole and cannot be delinked.

Towards sustainable development

As is clear from Figure 1 the very foundations of all sustainable or unsustainable development rest on the use or misuse respectively of the three capitals. The agricultural development can be regarded as a major solar enterprise backed by the use of some earth capital (land and water). The industrial development involves a major use of earth capital. Both agricultural and industrial developments are backed by human capital. While earth capital is finite and limited, solar capital is indeed clean and infinite. It has yet to be used to a significant extent. This can be a major input in improving chances for sustainability.

Furthermore, human mind has been very creative. During a relatively short span of time, it has created three types of diversity: agri-biodiversity, cultural diversity and developmental diversity (Figure 4). Agribiodiversity involved picking up the right kind of grasses and other plants and animals, followed by a meticulous domestication and selection process, so much so that it has not been possible for modern human being to add any totally *new edible economic species* even when powerful tools of science and technology have been at his command. One marvels at the meticulous work done by the so-called primitive human being. Based on the interplay of the three capitals, three types of human societies have evolved in a sequential manner.

Types of human societies

From the point of view of environment and development there are three types of societies. A *Hi-Tech or Throwaway Society and Economy*, a *Back-to-Nature or Subsistence Society and Economy*, and a *Sustainable Society and Economy*. The first two represent the two ends of the spectrum, while the third is indeed the 'middle path'. The Hi-Tech or Throwaway Society represents what one sees today in the industrial countries. They act as though the resources of the earth are unlimited and technology can help to do anything and everything. Such a society believes that developmental considerations are most important and subordinate to environmental ones. This pattern of life is unjustifiable and is not tenable on moral, ethical, economic and environmental considerations. The Back-to-Nature or Subsistence Society is regarded as primitive even though it may be sustainable. But it denies the fruits of modernity to the poor and the needy. This is unjustifiable and this segment has to be taken out of the morass of poverty and helped to enjoy atleast some of the fruits of modernity. In fact this is their right.

Sustainable society

Can one hazard a guess about the shape of a sustainable society in a country like India? Basically it has to be a healthy blend of environmental, developmental and economic imperatives. The underlying rationale has to be that ecosystems, agroecosystems and industrial economic systems have to be conserved and used in

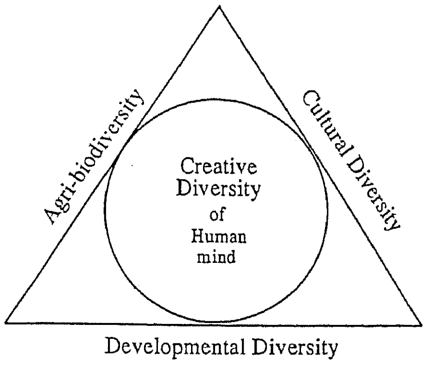

Figure 4. Creative diversity of human mind.

a sustainable manner. Furthermore, economic growth has not to be at the expense of ecological assets. The sustainable society has to aim at working in *partnership* with nature and conserve resources and energy, reduce waste, and avoid degradation of renewable resources. It must produce goods that are easy to recycle, reuse and repair after use. Sustainable economy aims at maintenance at a constant and sustainable level of both the number of people and quantity of goods. These should be in line with the carrying capacity of the concerned systems: ecosystems, agro-ecosystems or industrial economic systems. The basic needs of the people are met without any serious detriment to environment. *Need not greed, and comfort not luxury, should be the guiding principles.*

The method of growing food and raising livestock has to be based on soil and water conservation, bio-fertilizers, biological control of pests and minimal use of non-renewable energy. There is needed extensive use of relevant biotechnology under sustainable society. Under this path of development, people must believe that resources of earth have to be protected and sustained not only for human being, but also for other species. *The approach to manage and sustain resources of earth is not centered around human being but around the entire life-support system.*

Sustainable society and economy is based on a firm belief that earth is finite in area both for colonization of species and utilization of resources, but human numbers keep on growing. It follows from this that there cannot be infinite and unlimited growth and development with finite and limited resources that earth has. Furthermore, the increasing population growth and production and consumption of goods and services, stress and strain the natural processes and renewability so as to maintain the life-support system (air, water, soil, flora and fauna) in a healthy state. It is, therefore, essential that environmental degradation and depletion of resources is prevented by working *with* nature. The aim is to reduce unnecessary use and waste of resources (including energy) and not cause permanent extinction of species.

A sustainable society would largely be a solar/photosynthetic/biomass society where solar energy and solar hydrogen together with whole range of renewables are used and aims at not wasting resources unnecessarily and avoid interference with other species. The idea is to reduce short term gains that have long term environmental and economic costs.

A sustainable society would insist that the national accounting system should take into account both the economic growth rate and the rate of ecological resource degradation and rehabilitation. The two together will give a correct picture of the state of the country's economy. This would ensure that the economic growth is not at the expense of ecological assets. Although India has rather a rich resource base, the majority of the people are essentially poor. The sustainable group must have faith in science and technology as a powerful instrument of social and economic change and must advocate the use of technology relevant to a particular situation with emphasis on local self-reliance. As indicated above, we need to believe in recycling and reusing materials and advocate adoption of all the technologies that help to conserve the life-support system of the planet without affecting its regenerability. This would lead to rational use of resources with minimum waste. The guiding principle is to satisfy the need and not greed of the people, ensure comfort not luxury, and above all bring about equity with social justice. Unlike the Hi-tech Society, which basically in its present form works *against* nature, the Back-to-Nature Society works *in* nature, and the Sustainable Developmental Society would work *with* nature.

The attitudinal difference between eco-fundamentalists and sustainable developmentalists can be seen from the fact that the former talk of *ecocide*, *ecodisaster* and *eco-catastrophe*, while the latter, taking note of the former, talk and plan through *ecotactics*, *ecotechnology* and *ecodevelopment*. Their roles could be complementary to one another – the former use shock tactics to arouse interest, while the latter do something positive on the ground.

The twin goals of sustainable developmentalists are: restoration of the past ecological damage, and insulation of the country from the damage as a consequence of future development. The latter must entail minimum risk to environment. They recognize the fact that there is no form of development with zero-risk. To accomplish both restorative and preventive strategies, they advocate the use of science and technology in an abundant measure.

The most formidable task before a Sustainable Society is to achieve, in actual practice, sustainable development that will alleviate the condition of the teeming millions of India who have to be brought out of the present day morass of poverty, penury, want, illiteracy, disease and joblessness through the application of location-specific science and technology. It must firmly believe that our teeming millions in villages (including tribals and adivasis) are entitled to the fruits of modernity without affecting the resource base adversely. It is not ethical to keep them out of the mainstream of national development and advocate, as some eco-fundamentalists do, a Back-to-nature approach.

Panchayat Raj (governance through local Village Councils) shorn of its populist and political overtones, can be one major instrument of the much-needed socio-economic change at the grass roots for India's teeming millions. However, what we need is the right mix of development and environment to enable people to produce, protect and sustain resources so as to raise their quality of life. It would also generate employment and halt migration from villages to cities. The fundamental question is what pattern of development needs to be followed. The answer is simple; it has to be the bio-intensive form of development because foundations of our village society are biological: agriculture, animal husbandry, forestry and fisheries. These are all biomass-based vocations. Furthermore, there has to be intensification and diversification of biomass production, processing and utilization. To ensure this would involve sophisticated science and technology including biotechnology. Furthermore, biomass production has not to be monsoon-dependent. Such a positive approach alone will help rural people to insulate themselves against future ecological and economic shocks, which otherwise would make them 'ecological refugees'.

For the success of bio-intensive pattern of development at the grassroots, two measures are very necessary. Firstly, land use planning and land-tenure, which though somewhat intractable issues, have to be solved in favour of people particularly the weaker sections. Secondly, our per capita land holding is very small, and with population increase it would become still smaller. We have, therefore, to get more and more biomass from less and less land. This is possible by involving environmentally clean science and technology, particularly genetics, breeding, pharmaceutical sciences and biotechnology, and advocating use of not only high-yielding varieties but also biofertilizer and bioinsecticides in order to make biomass production sustainable and continuing to maintain the tilth and health of soil.

Our very life and existence and economic system depend on solar, earth and human capitals. The technology to use the earth capital is generated by the human capital. We have also to respect not only human life but affirm our responsibility towards all life.

Thinking must become systematic, holistic and futuristic. We must anticipate consequences of first, second and even third order, and must redesign our behaviour towards people, government and business, and above all towards our atmosphere, hydrosphere, lithosphere and biosphere. These should not be abused and over-used because these alone will help to evolve sustainable societies.

Models of development

Broadly speaking, there are two models of development for India (Figure 5). The top-down Nehruvian model of development involves industrial development in which mostly non-renewable resources (including energy) are used. Generally, such development everywhere has been oblivious of the destruction of natural resources, which represent wealth in their own right. The prime indicator of this development is the increase in Gross National Product. This is essentially a human-made macro-economic indicator, which neither reflects the extent and nature of human well-being, nor the damage done to the environment.

The bottom-up Gandhian model is basically aimed at building self-reliance and self-respect in a villager, and poverty alleviation of region's teeming millions who are steeped in penury. Village is a socio-economic and cultural unit and not a geographic unit. This model involves enhanced biomass production, processing, and utilization. A large section of our society has to be served by this model which depends on renewable resources (both man-made and natural). The model is largely fuelled by solar energy (photosynthesis). The indicator to be used for estimating growth of such a model has to be the increase in the Gross National Resource Product, which should be sustainable and should cause the least or manageable amount of ecological damage to the production base. The basic principles (local self-reliance and equity with social justice) of the Gandhian model of development must become applicable to all situations from ecosystem to industrial societies. However, the top-down model of industrial development is relevant primarily to the industrial economic sector. This model needs refinement and has to be made sustainable. The Gandhian model leads to decentralized economic planning and to an 'economy of permanence'[6] while in the top-down model of industrial economy there is the danger that the rich may become richer, and poor poorer. Success will be measured not by homogenizing a heterogeneous situation, but by harmonizing diverse societies in which modernity is appropriately blended with tradition, and where man-made capital does not become destructive of the natural capital. Both models have their specific constituencies. Thus, following a democratic path, *there is need for a creative synthesis of the bottom-up (Gandhian) and the top-down (Nehruvian) models.* Herein lies the salvation of this country.

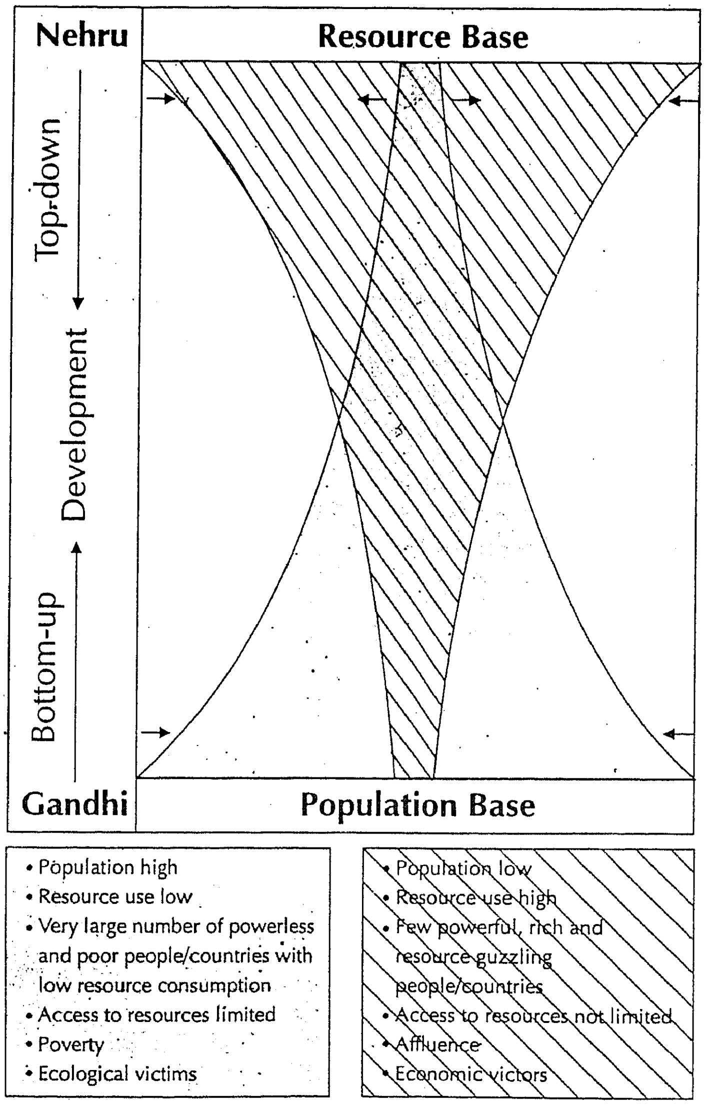
Nehru
Resource Base
Top-down
Development
Bottom-up
Gandhi
Population Base
• Population high
• Resource use low
• Very large number of powerless and poor people/countries with low resource consumption
• Access to resources limited
• Poverty
• Ecological victims
• Population low
• Resource use high
• Few powerful, rich and resource-guzzling people/countries
• Access to resources not limited
• Affluence
• Economic victors

Figure 5. Relationship between population and resource use in developing and industrial countries.

The two models are depicted diagrammatically in Figure 5. In one model a small percentage of population uses an unusually large amount of resources. Globally this is also true of a small number of powerful industrial countries guzzling resources far out of proportion. The other model results in a large percentage of the population using a small amount of resources, as is true of a large number of populous (but rather powerless) developing countries. Equalization between the two models can only be possible by shrinking the use of resources in the first group, while enhancing resources use and controlling population growth in the second group (Figure 5). At present, both these are only pious wishes because, for instance, the USA has about 5 to 6 per cent of the population of the world, but is guzzling about one-third of the world's non-renewable resources. From the resource-consumption point of view, its population actually constitutes over 20 per cent of the world's population. On the other hand, India has 16 per cent of the world's population, but from the point of view of actual resource-consumption, it represents less than 4 per cent of the population of the world[7]. The present situation neither reflects any form of equity nor social justice, and is indeed inherently unsustainable. It needs urgent attention, for otherwise it carries in it the germ of future confrontation between developing and industrial countries. *The advice from the latter to the former regarding controlling their population will carry conviction only when industrial countries give demonstrable proof of reducing their resource consumption*[2].

The best option for our region with its very large rural population is *bio-industrial development*, rather than pure industrial development. The bedrock of such development is sustainable production, processing and utilization of biomass (to meet the needs of the unusually large rural sector), together with a commensurate amount of pure industrial development. Furthermore, the GNP needs to be recalculated on the basis of depreciation or appreciation in land and soil, forests, water, biodiversity, fisheries, extent of climate change and ozone layer depletion, and so on. These calculations must also include specific indicators of human development and well-being. This is where India in the course of time, can blaze a new trail by appropriately blending economics and ecology into one connected whole. Herein lies the future of India in fostering pluralism and not singularism. This is both a challenge and an opportunity for our country.

It is clear that India cannot be against industrialism *per se* but it has to have industrialism minus its negative impacts, for example, labour displacement and exploitation and environmental degradation. The important characteristics of the two models are summarized in Table 1.

Today, a major challenge as also an opportunity before the country is how soon can we move towards sustainability. In India, if we go the way we have been so far, centuries will continue to co-exist in future. We will continue to have a subsistence India of a large number of poor and dispossessed toilers and plodders who live in medieval times, and an affluent India of a small number of people who are jet-set and wealthy who may be poised to enter the twenty-first century with a bang. How soon we take even the preliminary steps to bridge the vast gap between the large powerless subsistence and the small powerful affluent India, will determine whether we can make it to a sustainable society where we have environmental harmony; conservation of natural resources (including energy); economic efficiency; local self-reliance; gender equality; equity with social justice; ecological, social and economic security; sustainable consumptive ethic with cultural relevance; and peace and disarmament: the dream of Mahatma Gandhi[7,8].

The governments in the developing countries, for that matter even in the industrial countries, have yet to evolve sensible, credible and implementable agenda for ensuring sustainable ecological and economic growth, development and security. We need a comprehensive ecological and economic code about responsibility of an individual (because a society is actually an extension of individuals), society at large and country as a whole, and, to the extent possible, even of the South Asian Region. We also need to evolve a regional approach to the global issues which has become very important on account of our shared history, culture, religion, philosophy and above all fostered by our guardian, a healthy Himalaya, without which India would have been altogether different, may be a desert.

Tasks ahead

Economy–ecology nexus

There is a close connection between

Table 1. The two models of development

Bottom-up Gandhian model	Top-down Nehruvian model
Intensification and diversification of agriculture, animal husbandry and forestry, i.e. biomass production, processing and utilization, i.e. *renewable resources*	Intensification and diversification of industrial development using mostly *non-renewable resources*
Photosynthetic/solar model: use of solar and biomass energy and some non-renewable energy	Man-made industrial economic system: Use of non-renewable resources and energy. (This model should not be accepted without environment impact assessments and environmental management plans.)
Labour-intensive	Labour displacing
Caters to over 76% of population	Covers hardly 10% people
Poverty alleviation at subsistence level	Gap between rich and poor widening: rich becoming richer, poor poorer
Governance at village level through Panchayat (village council): Bottom-up approach	Governance centralized: top-down approach
Economy of permanence: Sustainable	Economy of impermanence: Unsustainable
Rural development	Industrial development

A creative synthesis of the two models is needed for achieving sustainable bio-industrial growth and development

Source: Khoshoo[7].

economy and ecology. The former emanates from the ecological assets (the earth capital) with the help of technology (the human capital). Economy and ecology are intimately interrelated, interconnected and interdependent. India presents to the Parliament an annual *Economic Survey*, and then a week later announces an Economic Budget. It is high time that we also present an Annual *Ecological Survey* of the country followed by an *Ecological Budget* with ecological deficit in the form of pollution and ecodegradation of water, air and soil, deforestation, etc. Following preventive and restorative strategies, we can wipe out ecological deficit, and have an ecological surplus in the form of clean air, water, soil and the increase in forest cover, sustainable agriculture, etc. We have to make ecology and economics as two sides of the same coin in real terms. Ecological and economic security are mutually reinforcing.

The major task facing the country is to set right the big environmental deficit created by past ecological damage, and to ensure manageable or no ecological damage from future economic development. *The most important point underlying sustainability is that we must live on the income (in the form of annual increment) from our life-support system and not on the capital.* With proper management it is possible to enhance the income. The idea is to evolve a portfolio of restorative and preventive environmental, social and economic strategies based on hard core science and technology. Examples of these are Ganga Action Plan, Wasteland Development[9].

Ecotechnology

Equally important is the realization that the future development is going to be through the use of ecotechnologies. In fact enlightened industrialists in Japan and Germany are increasingly switching over to such technologies on account of the realization that *there is going to be far more money in conservation technologies than in consumptive technologies.*

Urgent tasks

The most important tasks needing urgent attention are: population stabilization; land-use planning in our land hungry country; water conservation; sustainable agriculture, horticulture animal husbandry and fisheries; conservation and sustainable utilization of natural forests and raising large scale man-made plantations in order to save our natural forests; conservation and sustainable utilization of biodiversity; ecologically compatible housing particularly slum improvement; control of pollution of air, water and soil; non-polluting renewable energy systems; minimization, recycling and utilization of wastes; green technologies; control of AIDS epidemic; environmental education and training leading to environmental ethics; periodic updating of environmental laws; blending ecological and economic imperatives; and ethical and moral dimensions of resource use.

Attitudinal change

Finally, there has to be a major change in the attitude of the human race from purely techno(logical)-economical consciousness to a broader perspective of eco(logical)-economical consciousness[10], of which technology is one of the components. The important characteristics of the new environmental thinking are that it must become holistic, qualitative, spiritual, reverential, evolutionary and participatory. Herein lies the salvation of human being notwithstanding the fact that it may soon acquire the capability to clone itself, even so it must remember that *death is a reality*.

Role of human being

The foregoing tasks are more or less attainable, but human race has to take decisions about its future role. It is a part of the overall system which ranges from her/himself to universe in successively expanding horizons[11] (Figure 6). This may look to be beyond one's comprehension, but it imposes an implicit responsibility on human beings. Three things stand out from this diagram. Firstly, there is a continuum from one's own self to the universe. Secondly, there is a progressive dwarfening of the human being. Someone has put this idea differently: collect all the sand grains on the surface of earth, these will give some idea of the number of celestial bodies floating in the universe. Take just one of the sand grains, that would be our Mother Earth. Imagine one's own self standing on this grain: one among billions of people and countless other organisms (plants, animals and micro-organisms) living on the earth. Obviously, one feels humbled and miniaturized beyond recognition. Lastly, the fundamental point is that human being

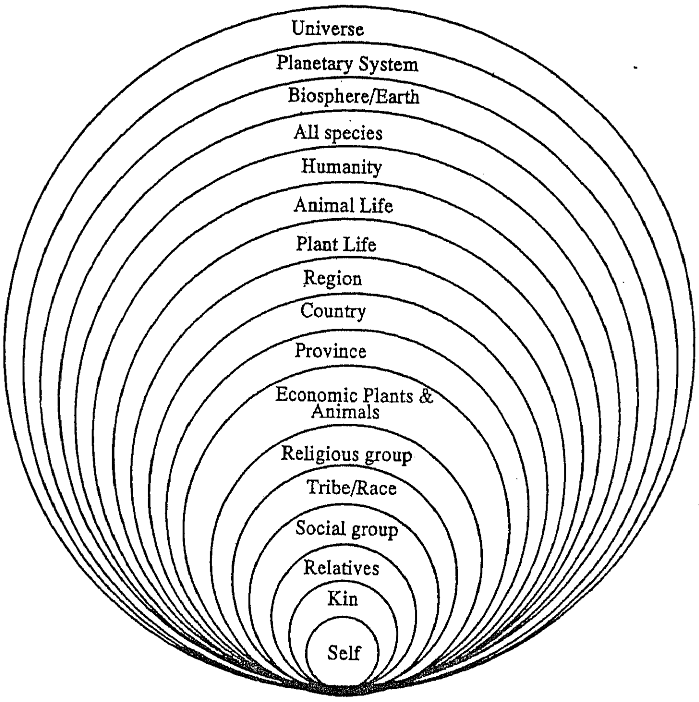

Figure 6. Place of human being in the universe. Modified from Noss[11].

must realize that it is not a co-creator. No doubt it is a species gifted to think, recollect and foresee, and added to this is the power of science and technology. This power must not be misused and abused. Therefore, human being must become a responsible species: scriptures talk of such a responsibility.

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Declining semen quality in Bangaloreans: A preliminary report

The antifertility effects of environmental pollutants have been known since Roman times when the lead content of drinking vessels was suspected to be the cause of declining populations in the upper classes[1]. A recent study in China has shown that exposure to low levels of lead causes an impairment of male fertility as evidenced by low volume of the ejaculate, low sperm concentrations and increase in incidence of nonviable spermatozoa[2]. Sperm counts in Parisians declines at a yearly rate of 2.1% in contrast to Frenchmen living in Toulouse who did not show any change[3,4]. The major difference between these two studies is that Toulouse is a rural area of France with a low population as well as car density and industrial pollution as compared with Paris. A drop in total sperm count has been reported in Greater Athens where there is an increase in air pollution[5]. More extensive studies carried out in Europe and the USA have shown that the human sperm concentrations as well as the incidence of morphologically normal and motile spermatozoa are progressively declining over the last few decades[6–10]. This decline has been attributed to air pollutants especially the xeno estrogens[11,12].

The purpose of this retrospective (1992 to 1996) study was to determine whether there was any marked change in semen quality in the 1625 men who had come for semen analysis to Hope Infertility Clinic, Bangalore. Semen data, viz. volume, sperm concentration and percentage of motile and morphologically normal spermatozoa, during these five years was correlated with changes for the same period in air pollution indices, viz. suspended particulate matter (SPM), sulphur dioxide and lead content. SPM refers to solid and semi solid material found in the atmosphere which are less than 0.1 μm in size. SPM is a complex mixture of soot, ashes, dirt, soil, dust, pollens, molds and other carbon-based particles and acid aerosols. Particulate pollution comes from wood burning, car exhaust, mining, construction activity, plants, changes in humidity and diesel emissions.

All semen analyses were carried out in the same laboratory using methods described in the WHO Manual[13]. Data was categorized as: azoospermia (absence of sperms); oligospermia (sperm concentration < 20 million/ml); asthenospermia (> 50% of sperms nonmotile) and teratospermia (> 50% of sperms were morphologically abnormal) and tabulated year-wise.

The average values of the major air pollutants: SPM, sulphur dioxide and lead for Bangalore were obtained from the Central and State Pollution Control Boards for the years 1992 to 1996 (Table 1).

The data was analysed using a Microsoft Excel software package. The mean volume of semen and the mean concentration of sperms in 1992 and 1996 was compared using the Student's *t* test. The relationship between the semen volume, sperm concentration and the average values of the air pollutants was measured by determining the correlation coefficient between the two variables.

Mean semen volume and mean sperm concentrations were significantly

Table 1. Mean semen volumes, sperm concentrations, incidence of oligospermia and SPM values in 1992 and 1996

Year	Semen volume (ml)	Sperm concentration (millions per ml)	Oligospermia (%)	SPM (μg/m^3)
1992 ($n = 410$)	3.5	69 + 2.97	25	141
1996 ($n = 118$)	3.0	43 + 3.67	35	245

($P < 0.05$) lower in 1996 as compared to those in 1992. The other most significant change observed was that the incidence of oligospermia increased from 25% in 1992 to 35% in 1996 (Table 1). There was no significant change in the incidence of azoospermia, asthenospermia, or teratospermia.

There was good inverse correlation between the ambient SPM concentrations on one hand and semen volume and sperm concentration on the other ($r = -0.8193$ and -0.83087 respectively for semen volume and sperm concentration vs SPM). A similar correlation could not be discerned between semen characteristics and ambient levels of sulphur dioxide and lead.

The results of this retrospective study, which indicate a decline in semen quality during the five years of study, are similar to those made in USA and Europe[3–10]. A significant finding is the inverse correlation between the mean semen volume and sperm concentration on one hand and SPM on the other as well as the rising incidence of oligospermia with a progressive deterioration of the environment. Bangalore is one of the fastest growing cities of Asia with a concomitant rise in the human population, motorized vehicular traffic and in the increased use of diesel generators – all of these contribute to the rise in total air pollution levels in the city. In addition to SPM, the organic and inorganic material that bind to it are substances well known to have detrimental effects on spermatogenesis and accessory sex glands.

The present study is far from comprehensive. Nevertheless, it clearly shows that semen volume, sperm concentrations and the incidence of oligospermia have drastically changed in such a manner that is indicative of reduced male fertility during the last five years. These findings give cause for concern about the altered reproductive health status of men *vis-à-vis* deterioration of environmental conditions. Spermatozoa are single cells and are likely to be the most vulnerable cells in the body to altered environmental conditions. If men are thus affected, surely women too would be similarly influenced by the environment. There is therefore an urgent need to carry out an in depth study of declining reproductive health parameters *pari passu* with environmental degradation. Such studies are underway in our Centre.

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Bambusa vulgaris blooms, a leap towards extinction?

BAMBUSA *vulgaris*[1] belonging to the tribe Bambuseae of Poaceae is the most widely grown bamboo throughout the tropics. Though described in 1810, the origin and nativity of this species is still debated[2–5], and it survives only in cultivation. Adaptability to different agroclimatic conditions, high culm strength, utility in various ways, high pulping quality, easy response to vegetative propagation, vigorous growth, quick recovery of clumps after felling and rare flowering are some advantages of this species.

The incidence of flowering in this species is very rarely reported. Blooming occurred in Bangladesh during 1851 and 1879, in Sri Lanka in 1863, India (Calcutta) in 1890 and Singapore in 1892 (refs 2, 6, 7). After the lapse of over a century, another flowering was reported in Bangladesh[8,9] during 1979, 1980–81 and 1983-84. Though not clearly indicated, Soderstrom and Ellis[4] studied flowering samples of *B. vulgaris* that flowered in Kandy district of Sri Lanka as late as 1970s. The flowering cycle is believed to be 80 (± 8) years[10]. During each occurrence of flowering only a few clumps were involved[3,8,11] and no report of gregarious flowering exists. Unusually, flowering was not followed by fruit setting in any recorded history and eventually clumps involved perished[3,8,10]. Banik[10], however, reported a clump which 'stopped flowering and revived'. Except a doubtful report by Lantican *et al.*[12] seeds of *B. vulgaris* remain to be botanically known[2–5]. As flowering does not result in fruit setting, it was subsequently doubted[8,9] whether this condition will lead to the eventual extinction of this unique species.

We observed, in May 1996, five clumps of this species in flowering in two private areas near the police station, Cherthala (9.42°N, 76.2°E), Alappuzha district in Kerala. (*Exsiccate*: 24 May 1996, *K. C. Koshy 28668, 28669*; TBGT). Incidentally, this is the report of its flowering from India after 100 years. Out of the five flowered clumps, culms in four were yellow with green stripes and the other with culms yellow (with green stripes) and green (with yellow stripes) together. Two clumps in one area were completely leafless and in full bloom (complete flowering[10]). No new shoots were produced from these clumps.

The efforts to find out fruits and seedlings were also futile.

In the other nearby area, 3 part-flowering[10] clumps were noticed. One clump was healthy and new sprouts were produced (culm sheaths were available). Flowers were produced only from branch complements up to 2–3 nodes above ground. In the other two clumps all culms were completely felled and new shoots were being produced from the stumps. Flowers were produced from branch complements arising from lower nodes of cut culms.

On a repeated visit to the same spot on 30 October 1996 after rainy season it was found that the two completely flowered clumps were felled. No seedlings could be located from the surrounding grounds. The other three partly-flowered clumps were healthy and produced new shoots. Flowers were seen on branch complements of old culms. To facilitate easy observation, offsets from part-flowering clumps were taken and planted in the bambusetum of Tropical Botanic Garden[13]. These offsets established in the field produced flowers for about 6 to 8 months and died without producing any seed. Artificial pollination conducted also did not yield any fruit. The florets were examined carefully to find out the possible physical barriers of sterility.

Spikelets usually contain 6 florets and a terminal rudimentary one. All florets often do not open. In many cases stamens of 3 florets come out and in other florets the sex organs do not attain maturity and dry while remaining inside the palea.

The palea which encloses the sex organs is two keeled with wing-like margins infolded and sulcate between keels. The keels are ciliate on upper portion. The palea is closely overlapped by lemma. At the time of opening due to the pressure of well developed and massive anthers, lemma and palea widen at tip keeping a gap of 1.5–2 mm through which the stamens come out. Once the stamens emerge out, lemma and palea close back to its original position (Figure 1 *a*).

The gynoecium is found to be 'unhealthy' and falls under three categories. The length of entire gynoecium is (a) less than the length of palea. This is observed in majority of cases; (b) as long as palea

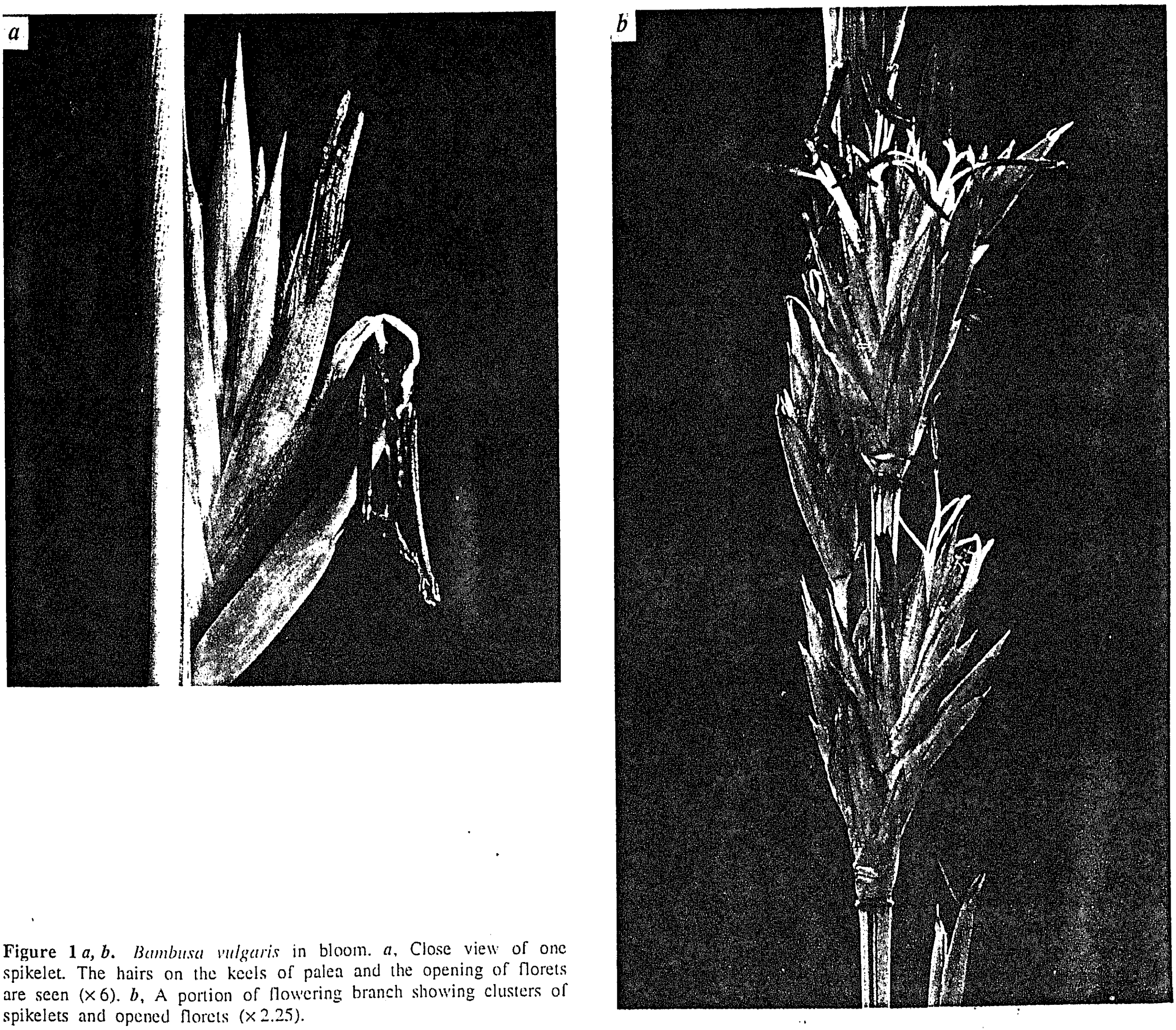

Figure 1 *a, b*. *Bambusa vulgaris* in bloom. *a*, Close view of one spikelet. The hairs on the keels of palea and the opening of florets are seen (×6). *b*, A portion of flowering branch showing clusters of spikelets and opened florets (×2.25).

or (c) slightly longer (middle stigma only) than lemma as in well developed ones. Only in such cases at least a portion of stigma comes out on opening of floret.

The stigma is usually two in number, the third one is not well developed. The entire stigmatic portion is only 3–4 mm long. Tip of middle stigma of 1.5–2 mm struggles, along with stamens, to come out when lemma and palea widen. Rest of stigma remains inside the closed floret. The receptive stigmatic portion exposed to receive pollen is thus very limited.

The bristle-like hairs of palea (Figure 1 *a*) are longer than the stigmatic hairs and in an opened floret the exposed but small stigmatic portion is often covered (?) by the hairs on the two keels of palea. Possibly this can act as a barrier preventing stigma from receiving the pollen grains.

Moreover, the reported[10] higher percentage of sterility (70–92%) of pollen may also contribute to the sterility in *B. vulgaris*.

The available evidence points to the imminent danger of extinction of this mysterious species due to (i) the death of clumps after flowering, (ii) the lack of fruit set, (iii) the inherent 'unhealthy' nature of stigma to receive pollen, (iv) the possible role of bristle-like hairs as barriers preventing pollination, and (v) the high pollen sterility.

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Restriction fragment length polymorphisms of the rRNA genes in some pulses

Recognition and exploitation of variations among genetically divergent groups of germplasm are fundamental in breeding and genetic engineering programmes. Restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNAs (RAPDs), DNA finger printing, inter simple sequence repeat amplification (ISSR) and amplified fragment length polymorphisms (AFLPs) are powerful tools for studies of plant genetics, evolution, germplasm diagnosis and crop improvement[1–4]. These techniques allow a direct analysis of the plant genome at the DNA level. RFLP analyses have been used as molecular markers to construct linkage maps of crop plants, to mark quantitative trait loci and to complement phylogenetic relationships in several plant taxa[5–7]. rRNA genes, although not with the same impact as chloroplast DNA, have proven to be of tremendous utility in phylogenetic reconstruction[8]. They also provide valuable genetic markers for the analysis of genomic relationships among cultivated species and their wild relatives[9]. The tandem arrays of rDNA repeat units, generally located in the nucleolar organizing regions (NORs) of chromosomes, combine highly conserved gene regions, encoding rRNA, with more variable intergenic spacer regions (IGS). IGS regions, which separate the adjacent transcription units, have been found highly variable in sequence, length and copy number of subrepeats in several plant genera[10]. Because rRNA gene sequences are subjected to relatively rapid rates of concerted evolution[11], they produce DNA fragmentation patterns that are highly homogeneous within individuals and among closely related populations or species, yet exhibit characteristic heterogeneity between groups. In comparison, single copy gene markers[12] often tend to exhibit as much within-group as between-group variation in plant species. rRNA polymorphisms can, therefore, constitute useful genetic markers[9,13]. To achieve a better understanding and to provide molecular evidence for the systematic relationship between and within some populations of *Lablab*, *Dolichos* and *Vigna* species we investigated the polymorphism of the rRNA genes.

Five populations of *Lablab purpureus*,

one population each of *Dolichos trilobus*, *Vigna bournii*, *V. grahmiana*, *V. unguiculata* and *V. wightii* were used for the study. These natural populations were collected from Pulney Hills of Western Ghats, Tamil Nadu, India. The voucher specimens are deposited at Rapinat Herbarium, Tiruchirapalli (RHT), India.

After sacrification seeds were soaked in water overnight (10–16 h), rinsed thoroughly and grown on wet vermiculite under a light bench (10,000 lux, day/night cycling: 16 h/8 h). After three days the seedlings were planted in pots containing garden soil and grown. After 30 days, the primary leaves were harvested, frozen with liquid nitrogen and stored at –70°C until DNA extraction.

Total plant DNA was isolated and purified using a combination of the maize DNA miniprep method[14] and the cetyltrimethyl ammonium bromide (CTAB) method[15], as generally suggested[16]. The yield of DNA was 30–100 μg g^{-1} tissue; the UV absorbance ratios at 260 nm/280 nm and 260 nm/230 nm, were at least 1.9 and 2.1 respectively.

All DNA samples (5 μg each) were digested with *Eco*R1 and *Bam*H1, (5 units per μg DNA) in buffers recommended by the manufacturer (Boehringer Mannheim) at 37°C overnight. Electrophoresis was performed following standard procedures[17]. The molecular size marker Raoul-1 (Appligene, France) was used. Capillary blotting of the gels to nylon membranes (Hybond N, Amersham) was done according to the instructions of the manufacturer. A 3.5 kb *Bam*H1 fragment of the genomic clone PTA 250 (ref. 18) was used in this study. It was labelled with digoxigenin (Boehringer Mannheim). Prehybridization, hybridization and washing of the membranes were done following usual methods[17]. Detection was done nonradioactively with chemiluminescence. For autoradiography, the X-ray film (X-OMAT AR, KODAK) was exposed to the membranes between two intensifying screens (Lighting-Plus, Du Pont) at –70°C for 5 h.

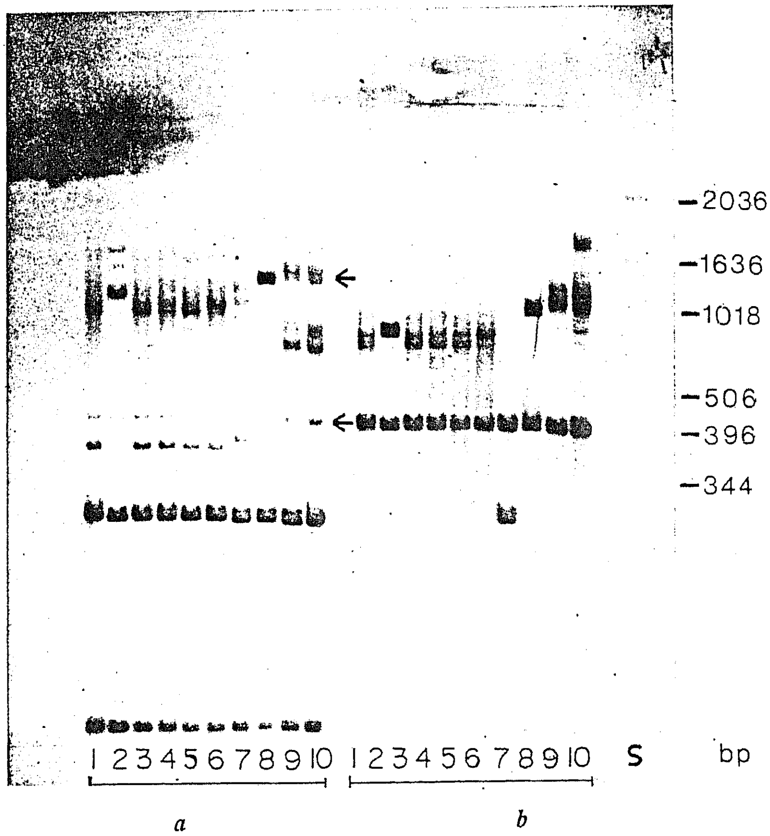

Figure 1. 1. *Lablab purpureus* population 1; 2. *Dolichos trilobus*; 3. *Lablab purpureus* population 2; 4. *Lablab purpureus* population 3; 5. *Lablab purpureus* population 4; 6. *Lablab purpureus* population 5; 7. *Vigna bournii*; 8. *Vigna grahmiana*; 9. *Vigna unguiculata*; 10. *Vigna wightii*; S = Standard Marker. *a* = *Eco*R1 digested RFLP patterns. *b* = *Bam*H1 digested RFLP patterns. Arrows = intergenic spacer region.

Inter-generic, inter-specific and intra-specific polymorphism could be detected with the two endonucleases used in this study (Figure 1 *a* and *b*). Polymorphisms between species are higher than polymorphisms within species or between genera. The results show that only a few polymorphisms could be detected within the coding regions of the rRNA genes of *Lablab purpureus* whereas there were greater number of polymorphisms between species and between genera. These data correspond with results found in other plants[19].

The hybridization patterns of five populations of *Lablab purpureus* showed great similarity. The hybridization patterns of four different species of *Vigna* were very dissimilar. The hybridization patterns of the three different genera, namely *Lablab*, *Dolichos* and *Vigna* were also quite dissimilar. Our RFLP analysis supports the taxonomic classifications[20,21]. The appearance of clear bands suggests a limited number of loci, each containing a small or large number of repeats.

The present study on rRNA genes reflects the relatedness among genomes of the *Vigna* species with reference to the restriction sites.

Our results indicate that RFLPs of the rRNA genes are helpful in detecting inter-generic, inter-specific and intra-specific variations and thus contribute to an understanding of taxonomic relationships.

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Height vs water depth for small sand ripples – An aid to palaeohydraulics

Water flowing over a plane non-cohesive sediment surface throws it into a series of rhythmic bedforms which are designated as ripples (height < 0.075 m) and dunes (≥ 0.075 m) in lower flow regime. The nature and magnitude of these bedforms of lower flow regime are dependent on parameters like flow velocity, water depth and sediment grain size. The relationship between dune height and water depth has been worked out by Allen[1], $H = 0.086 \cdot d^{1.19}$ (H being the dune height and d, the corresponding water depth). Using some well-known hydraulic relationships in proper sequence, attempts are made to determine the flow velocities and mean annual discharge for ancient streams from dune heights preserved in rock sequences[2–4] (see ref. 5, pp. 279–280 for details).

The dimensions of the dunes used by Allen for establishing the above-mentioned relationship are large (H varying between 0.10 m and 10 m), but the ripples encountered in ancient sediments (rock record) are often of much smaller dimension. A series of experiments were conducted in a 'close circuit' hydraulic channel using sand grains of two different size ranges (Table 1) to determine the relationship between small ripple height and water depth. The heights and wavelengths of the ripples generated over these sand beds at a fixed flow velocity (~ 0.30 m/s) but varying water depths (0.088–0.185 m above the sand bed) ranged between 0.009–0.0165 m and 0.17–0.32 m respectively. The relationship between water depth and ripple height obtained from these experiments is $H = 0.065 \cdot d^{0.82}$, d being the effective water depth above the ripple crest (Figure 1). This relationship, somewhat different from that obtained by Allen, is expected to provide a realistic clue to palaeohydraulic parameters when small ripples are involved. Obviously, this relationship is valid for small water depths only. Although Allen used water depths as large as 10 m, in great water depths (as in oceans) ripple height may be independent of water depth.

Table 1. Grain-size of the sands used in the experiments

Sand no.	Mean grain-size (mm)	Standard deviation (mm)
IIT-1.1 (*)	0.42	0.287
IIT-4.1 (+)	0.175	0.052

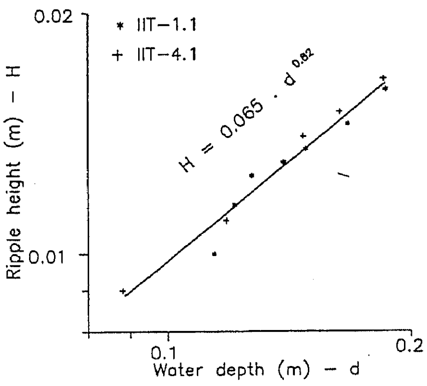

Figure 1. Water depth vs ripple height for small ripples.

The dimensions of subaqueous bedforms are known to be dependent not only on water depth, but also on flow velocity and grain size. The ranges of grain size and flow velocity for the data compiled by Allen are not explicitly stated in his publication. In the present case the grain sizes of both the sand samples used for the experiments as also the flow velocity lie within the lower flow regime ('ripple field' of Southard and Boguchwal[6]), thereby eliminating the possibility of distortions arising out of grain size or velocity variation.

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Disease-resistant genes: New approaches towards the control of parasitic diseases

Populations infected with various parasites have been estimated as 270 million for malaria, 200 million for schistosomiasis, 90 million for filariasis, 18 million for Chagas diseases and 12 million for leishmaniasis[1]. Drug research is a top priority in many countries though drug use has many disadvantages. The various drugs and their toxic effects toward host system make drug use a threat to humans. Drug researchers are aware of the side effects and increasing drug resistance of the pathogens.

Recent studies have resulted in remarkable findings in identifying several disease-resistant loci in mice. These are identified as *Lsh*, *Ify* and *Bcg*, denoting resistance to *Salmonella typhimurium*, *Leishmania donovani*, *Mycobacterium bovis*[2–5]. These genes are located on the chromosome 1. Later studies have identified and selected several such genes in mouse[6–10] for a variety of pathogenic infections (Table 1). How these genes confer resistance towards pathogenic infections is not known. Cloning of these genes and their expression in other mammalian systems will give an understanding of how to combat parasitic diseases. Even though the hypothesis of one-gene : one-disease for hereditary diseases cannot be applicable in this context, one can think of polygenic localization of such disease-resistant factors. Recent progress in the isolation and characterization of candidate genes and new microsatellite-based markers for family linkage studies of the whole genome is at its frontiers. However, genome scanning approaches to gene mapping for infectious diseases are in preliminary stages in the case of parasitic diseases. This approach will be a fair play to overcome parasitic diseases and to tackle problems of drug use and its associated hazards.

Table 1. Various genes/loci identified in mammals have impact on pathogenesis resistance or susceptibility

Gene/locus	Chromosome	Function encoded	Disease/pathogen
MHC class II	17	Membrane glycoprotein presents antigen to CD4$^+$ T cells	Malaria Leprosy
MHC class I	17	Membrane glycoprotein antigen to CD8$^+$ T cell	*T. gondii*
MHC class I-b	17	Non classical MHC antigen presentation to CD8$^+$ T cell	Listeria
TAP	17	Peptide transporters located in ER	Intracellular infections
LMP	17	Proteasomes: degradation of protein, antigen representation	Intracellular infections
Bcg/Ity/Lsh	1	Enhancement of APC function	*Mycobacteria, Leishmania Salmonella*
Vβ5	6	Response to superantigen	*T. gondii*
Vβ6	6	Response to viral superantigen	Polyoma virus
Vβ8	6	Response to superantigen	Staphylococcus, *T. gondii*
VI(γ)δ subset	13	Response to Mycobacterial super-antigen	*Mycobacteria, Leishmania*
TNF-α	17	Glycoprotein	*T. gondii*, Malaria
IL-4	11	Prototypic Th2 cytokine	*Leishmania*, Leprosy
INF-γ	10	Th1 cytokine	*Leishmania, Mycobacteria*
Cryp	8	Cell defense	*T. gondii, Salmonella*
NOS2	11	No synthase; control pathogen multiplication	*Mycobacteria, Salmonella Leishmania*
Mx 1	16	75 kDa Mx 1 protein	Influenza
Anth 2	2	Control pathogenesis	*Bacillus anthracis*
Bcga	12	Anergy to DTH	*Mycobacterium bovis*
HC	2	Pulmonary clearance of bacteria	*Staphylococcus aureus*
Lsr I	2	Bacterial multiplication	*Listeria monocytogenes*
Rict (Era 1, Api 1, Flu 1)	5	Control pathogenesis	*Rickettsia tsutsugamushi*
Ld	17	Brain parasite burden	*T. gondii*
Sc 11	8	Resolution of cutaneous lesions	*Leishmania tropica major*
Sc 12	4	Resolution of cutaneous lesions	*Leishmania maxicana maxicana*
Cmv 1	6	Virus replication in spleen	Cytomegaloma virus
Fv 1	4	Splenomegaly	Friend virus
Fv 2	9	Splenomegaly	Friend virus
Hv 2	7	Infection of macrophages and neurons	Mouse hepatitis virus 4
Rmc 1	1	Infectively retardation	Mink cell focus forming virus
Rmc f	5	Infectively retardation	Mink cell focus forming I
Rmp 2	2	Survival	Mouse pox
Tmevd 1	6	CNS demyelination	Theiler's encephelomyelitis virus
Tmevd 2	3	CNS demyelination	Encephelomyelitis
Rmv 1, 2	1	Viremia	Malaney virus I

Mouse genes and their location on chromosomes have been described, however, human homologous genes have also been identified and many more will be identified within coming decades.
CNS, central nervous system; ER, endoplasmic reticulum; T, *Toxoplasma*.

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Allelochemical synergism and insect behavioural diversity

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Synergism in insect–plant interactions plays a pivotal role in increasing the bioactive efficacy of allelochemicals and also influences the behavioural diversity of insects. As herbivore-induced plant volatile blends act as communication signals to natural enemies, the role of synergism in tritrophic interactions is very significant. Pheromonal synergism being widespread in insects, the precise composition of the pheromone stimulus affects their orientation behaviour, different isomers tending to evoke diverse behavioural patterns.

RECOGNITION of plant allelochemicals as agents influencing the behaviour of insect herbivores and their natural enemies is comparatively recent. The green leafy odours of plants, comprising mixtures of fatty acid derivatives such as C_6 aldehydes, alcohols and esters provided by the lipoxygenase pathway, form 'aerial bouquets', enabling phytophagous insects to reliably detect host plants. For instance, volatiles influencing searching behaviour include (Z)-3 hexanol, (Z)-3 hexenal, (E)-2 hexanol, (E)-hexanol and (Z)3-hexanyl acetate[1]. This reliability and detectability of volatile chemical stimuli from the first to the second trophic levels are of considerable significance. Subsequent feeding by phytophagous insects cause qualitative or quantitative changes, resulting in specific or nonspecific, direct or indirect modifications through alteration of their carbon/ nutrition balance. Being of short-term duration, detectability of these compounds often poses a problem unlike those of the more durable blends of terpene volatiles emitted from insect-damaged plants. While different host species may cause differential release of plant compounds, synergism has come to play a pivotal role in increasing the bio-active efficiency of the allelochemics. In many cases, systemic responses induced by insect feeding are mediated by elicitors that are transported from the infested to the uninfested sites. Because plant volatiles are known for their diversity of chemical components as well as their changeability in time, natural enemies become exposed to the mixture of compounds, enabling synergistic interactions. Besides, blends of pheromones cause sequential behavioural patterns and a highly adaptive, typically synergistic behaviour is evident in ants and other social insects.

Emphasis has been laid on the need for increased realization of the role that synergism plays in insect–plant interactions as well as tritrophic interactions. This will go a long way in pinpointing the compound(s) responsible for the behavioural diversity of insects. Of equal significance is the recognition of synergistic interactions between viruses and chemical pesticides and between viruses and other pathogens which have reduced the rate of application of chemical insecticides. Plant natural products influencing the efficacy of entomopathogens are known, as evidenced by the action of chlorogenic acid and polyphenol oxidase. These alter pathogenecity by acting directly on Btk[2].

Synergistic reaction of allelochemicals

Synergism is usually coalitive wherein a system of two components of which each alone causes no remarkable impact, but together provide a catapulting effect. A notable instance is gossypol, a phenolic sesquiterpene toxic to a variety of insects. It causes significant decrease in the survival, growth and development of several cotton pests. Pigment glands of cotton plant produce another sesquiterpene, caryophyllene oxide[3], serving to synergize the growth-inhibiting effect of gossypol. Increased vigour of the boll weevil is evident through ingestion of gossypol, which suppresses the growth of gut bacteria. An enhanced phagostimulatory effect is seen when sinigrin and glucose interact as evident in *Plutella xylostella*. Similarly piperonyl butoxide is known to be an effective synergist in conjunction with diflubenzuron in the case of *Spodoptera exigua* increasing the toxicity of diflubenzuron[4].

Synergistic effects of gallic, vanillic and salicylic acids are evident not only in their interaction with other plant allelochemicals, but also in their interactions with the endotoxins of *Bacillus thuringiensis*. Gallic acid occupies an important position in the overall phenolic metabolism of higher plants exhibiting synergistic interactions. Interestingly enough, majority of hydrobenzoic acids (*p*-hydroxybenzoic, salicylic, vanillic, syringic, protocatechuic) are relatively rarely encountered in plants, particularly in the herbaceous dicots. Various cinnamic acids (*p*-coumaric, caffeic and synapic acids) are ubiquitous in the tissues of higher plants formed normally as monoesters and rarely as bi-esters[5] (Figure 1).

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Effect of the addition of allelochemicals and/or entomocidal toxins in the diet, on the food consumption patterns of *Helicoverpa armigera* larvae showed a reduction from 331 mg/day to 92 mg, 79 mg and 56 mg/day by supplementation with gallic acid, βt endotoxins and resorcinol respectively. At higher concentrations of endotoxins with gallic acid, the feeding rate was reduced to a greater degree[6]. Similarly the impact of vanillic and salicylic acids on the development, survival and adult emergence of *H. armigera* also varied greatly with the concentrations and combinations tested, as compared to individual phenols[7].

Demonstration of synergism between two natural products, β-asarone and menthol has shown the involvement of enzyme induction. Bioactivation of β-asarone is synergized by menthol, an MFO inducer, synergism resulting from metabolic bioactivation by an enzyme[8]. Similarly, toxicity of xanthotoxins to *Heliothis zea* caterpillars has been known to be increased by myristicin. Other synergists like safrole and apiole occur alongside with xanthotoxin in Umbelliferae as also fagaramide, asaranin and sesamin in Rutaceae. The occurrence of these compounds at sublethal concentration suggests their having a primary role as phytosynergists[9].

Synergism in tritrophic interactions

Equally relevant is the fact that a number of plants damaged by insects emit chemical signals in large amounts that guide natural enemies to the concerned insects. To be effective, the emitted volatiles should be clearly recognized from the background odours, often coinciding when natural enemies forage. Besides, the green leaf odours mentioned earlier, after several hours of feeding result in the release of compounds such as linalool, (3-E)-4,8,dimethyl-1,3,7-nonatriene, α-trans bergamotene, β-farnesene, (E)nerolidol and (3E,7E)-4,8,12 trimethyl 1,3,7,11 tridecatetraene and indol. The insects are able to recognize differences in the ratio between these green leafy compounds[10]. Programming or imprinting to specific plant environments is typical of some parasitoids. Some compounds are short range ones, not very volatile, but requiring high concentration for eliciting responses. Related strains or chemotypes of a given plant also tend to differ in attraction if their volatile chemical profiles are different. However, a combination of cues elicit better responses by female successful parasitoids[11]. The mites *Tetranychus urticae* and *Panonychus ulmi* are known to affect several host plants, including apple leaves and release volatiles after feeding, eliciting different responses from predatory mites. While the relative percentages of chemical constituents differed between blends of volatiles, two compounds, viz. (E,E)-α-farnesene and (Z)-3-hexan-1-yl-acetate, with farnesene make up more than 50% of the blend[12]. This confirms that plants are the major sources affecting variation in chemical information available to natural enemies. Thus the environmental effects of allelochemical variation become important.

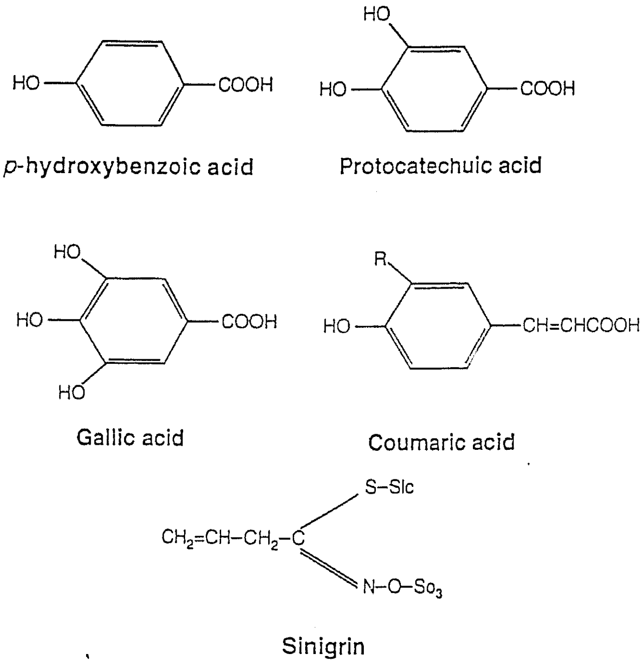

Figure 1.

In tritrophic interactions, herbivore-induced, carnivore attractants play an important role. Herbivore caterpillars regurgitate salivary β-galactosidase[13]. This elicits plant response, usually in the form of release of volatiles which increase host detectability by natural enemies. Herbivore-induced plant volatiles provide natural enemies with information about plant species, plant cultivars and the herbivore insect species infesting the plant. Thus the herbivore-induced volatiles are essential information source for natural enemies and act as communication signals[14]. In spite of these, our understanding of the role of plant odours in phytochemical-insect responses is very limited. It is clearly evident that the overall function of volatiles in plant–insect interactions is to act as chemical signalling systems with volatile blends promoting efficacy of interactions. Both quantitative and qualitative differences in the blends of these volatiles play important roles in such functioning.

Pheromonal synergism

Sex pheromones are single, two component or multicomponent chemical blends, with chain lengths varying from C_8 to C_{18}. They occur in different proportions with several other chemicals and are present in a range of related and unrelated species. The precise composition of the pheromone stimulus affects orientation behaviour, and different isomers can evoke very different behavioural patterns. Such blends as Z(9) and Z(11) tetradecenyl

Table 1. Examples of pheromone mixtures of well-known Indian insect pests showing synergism enabling increased sexual responses

Agrotis ypsilon	(Z)-11-dodecenyl acetate (Z)-9,tetradecenyl acetate
Chilo suppressalis	(Z)-11-hexadecenal (Z)-13-octodecenal
Helicoverpa armigera	(Z)-11-hexadecenal (Z)-9-hexadecenal
Pectinophora gossypiella	(Z,Z)-7,11-hexadecadienyl acetate (E,Z)-7,11-hexadecadienyl acetate
Phthorimaea operculella	(E,Z),4,7-tridecadienyl acetate (E,Z,Z)-4,7,10 tridectrienyl acetate
Plutella xylostella	(Z)-11-hexadecenal (Z)-11-hexadecenyl acetate
Spodoptera litura	(Z,E)-9,11,tetradecadienyl acetate (Z,E)-9,12 tetradecadienyl acetate

acetate in the ratio of 1 : 3 are common. Often the blend produces a greater response than any individual component, and this synergism is widespread in insects that produce pheromone 'cocktails'. When the blend comprises of four components, males are attracted to more complex ratios such as 60 : 40 : 2 : 200, when the components are (Z) and (E)-11-tetradecenyl acetate, (Z)-9-tetradecenyl acetate and dodecenyl acetate[15]. The proportion of the components varies according to the abundance of the species. Certain parasitic tachinids and braconids and other parasitoids locate their hosts by tuning to their (hosts) long distance sex-attractant pheromones.

Synergistic effects of terpenoid mixtures on bark beetles are well known. Frontalin of males synergizes with exobreviconin of females and myrcene sequestered directly from oleoresin of trees, to produce a rich 'pheromone bouquet'. The cumulative lure of all the three pheromones is synergistic, causing aggregation of beetles[16]. Many lepidopteran pests have aldehydes and ketones as sex pheromones. Some pheromonal compounds of *Helicoverpa zea* such as hexadecanal, (Z)-7 hexadecanal, (Z)-9-hexadecanal and (Z)-11 hexadecanal influenced the host-seeking behaviour of *Trichogramma pretiosum*. The application of a blend of these chemicals increased parasitism rates in treated plants[17]. Table 1 provides the composition of some sex pheromones of major lepidopteran pests in India[18]. In *Callosobruchus chinensis* a mixture of methyl-branched saturated hydrocarbons and (E)-3,7,dimethyl-2-octene-1,8-dioic acid elicit copulatory effects of males, the copulation releasing hormone being erectin[19,20]. The combination of a highly stimulating excitant like formic acid and a direction stimulus, like undecane, results in excitement in ants which rapidly 'home in' very accurately. A combination of two hydrocarbon with formic acid releases a more intense response than one hydrocarbon. In *Myrmica rubra* the primary alarm pheromone 3-octanone is an attractant, while 3-octanol is an arrestant, together producing klinokinesis in excited workers[21]. Pheromonal synergism which incites sequential behavioural responses, is much evident in several insects.

Briefly, there is no dearth of information on allelochemical synergism. Besides, augmenting the resistance mechanisms in plants against insects, the role of allelochemical synergism in tritrophic interactions is essentially to enhance the behavioural diversity of natural enemies, enabling more efficient biological control. Pheromonal efficiency through regulation of population build up of several agricultural pests by intercepting the communication signal between sexes is an added feature. With biopesticide industries cropping up rapidly, recognition of synergistic interactions should lead the way towards augmentation of the efficacy of the bio-active compounds, notably plant-based or *β*t-based products. BUGSAC[22], a herbal preparation made from 46 plant extracts such as neem, pepper, tulsi is a notable instance, acting as a nontoxic, ecofriendly pesticide effective on several pests, contributing to synergistic trends in the chemical amalgam.

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Gamma-ray diffraction – A powerful tool in crystal physics

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In recent times, gamma-ray diffraction has emerged as a powerful tool in structural and defect studies of crystals. Though similar in principle to X-ray and neutron diffraction, the very short wavelength of γ-rays gives gamma-ray diffraction an edge over the other diffraction techniques. Experimental details and several applications are discussed.

THE discovery of X-ray diffraction (XRD) by Laue in 1912 opened up two branches in physics, viz. X-ray spectroscopy and X-ray crystallography. Tremendous strides were made in both these branches in the decades that followed. Neutron diffraction (ND) which was discovered in 1936 also developed as a complementary technique in structural studies. Important aspects of XRD and ND are discussed by Bacon[1].

Gamma-ray diffraction (GRD) was demonstrated by Andrade and Rutherford (see ref. 2) in 1914. However, unlike XRD, the application of GRD remained confined to the measurement of γ-ray wavelengths[3] for quite some time and it was only in the seventies that GRD emerged as a powerful tool in crystal physics and has been used to advantage for tackling several problems for which the other diffraction techniques were not so effective.

The purpose of this article is to discuss (i) the principle and special features of GRD *vis-à-vis* other diffraction techniques, (ii) the experimental details, and (iii) some typical applications.

Principle and special features

Gamma rays are scattered by electrons associated with the atoms in the crystal and, as such, the principle of GRD is basically the same as that of XRD. GRD is governed by Bragg's law and the expression for the intensity (or reflectivity) of a gamma ray Bragg reflection is the same as that for X-rays[4]. Fourier procedures developed for XRD are applicable to GRD and lead to electron charge distribution.

However, there are some significant differences between XRD and GRD and some special features associated with GRD mainly due to the very short wavelength of γ-rays; these are discussed below[5–10].

i) The short wavelength of γ-rays results in negligibly small absorption. This makes the absorption correction to intensities very small and insignificant.

ii) Another consequence of the low absorption is that thick crystal samples can be used without surface preparation and, further, the presence of ovens, cryostats and pressure devices does not introduce any experimental complications.

iii) The short wavelength makes extinction effects negligible and if at all a small extinction effect is present, it can be corrected in a comparatively simple manner.

iv) The energy of γ-rays being much larger than the binding energy of the inner electrons, anomalous dispersion cannot take place and the corresponding anomalous dispersion correction becomes unnecessary.

v) As will be explained in detail later, because of the short wavelength, the width of the GRD rocking curve is determined only by lattice tilt (mosaicity) and the strain contribution vanishes.

vi) Though γ-ray sources emit several radiations, each γ-ray line is monoenergetic. Each wavelength can be selected by means of a single channel analyser. Hence, monochromators are not required.

vii) With the availability of intrinsic Ge detectors for measurement of GRD intensities, the peak-to-background ratio is very high, obviating the need for background subtraction which is a major source of error in XRD.

viii) γ-ray sources follow the well-known radioactive decay law and consequently there are no random fluctuations in the intensity.

ix) By measuring transmitted and diffracted intensities, absolute structure factors are determined. As mentioned above, a number of corrections which are unavoidable in processing XRD data do not occur in GRD and, hence, the determination of absolute structure factors can be done with a high degree of accuracy (~ 0.5%). The accuracy is often limited by non-diffraction factors like limits of error in counting statistics or error in measurement of crystal thickness. Such accuracy is not possible with XRD.

x) For the sake of completeness, two problems arising out of the short wavelength of γ-rays may be mentioned. Firstly, the entire diffraction pattern is shifted to very small Bragg angles. Secondly, the short wavelength gives rise to the phenomenon of multiple Bragg scattering which results in a systematic error not present in XRD. However, this undesirable effect is negligible up to $[\sin\theta/\lambda] \leq 0.8$ but for larger values of $[\sin\theta/\lambda]$, a correction becomes necessary.

Experimental details

The main components of a γ-ray diffractometer are the source, the collimators, the goniometer and the detectors.

Several radioactive sources have been tried like ^{198}Au, ^{192}Ir, ^{169}Yb, ^{160}Tb, ^{153}Sm and ^{137}Cs. The relevant properties of some sources are summarized in Table 1. The criteria for the choice of optimum source are specific activity, neutron and γ-ray self-shielding, source half-life and wavelength (energy) of the radiation. Because of their short half-lives, the ^{198}Au and ^{153}Sm sources need repeated irradiation by thermal neutron flux and hence diffractometers employing these sources have to be installed very close to nuclear reactor stations. In contrast, with the ^{192}Ir and ^{137}Cs sources, which have larger half-lives, it is possible to install γ-ray diffractometers even in laboratories away from nuclear reactors.

Schneider[11] has discussed the design of the γ-ray diffractometer in use at the Laue-Langevin Institute at Grenoble in France. The plan of this diffractometer is shown in Figure 1 *a*; the design of the goniometer (Figure 1 *b*) and source holder (Figure 1 *c*) are also shown. This instrument uses an irradiated gold foil as the source. In the diffractometer set up at the Hahn-Meitner Institute at Berlin (Schneider)[12], there is provision for using either a gold source or an Ir source.

γ-ray wavelengths are 10 to 50 times smaller than X-ray wavelengths. The result is that whereas in XRD, measurable Bragg reflections occur in the angular range of 3°–89°, in GRD, the Bragg reflections occur at very small angles in the range 5′–5°. Thus, the GRD experiment is essentially a small angle scattering experiment. Hence, collimation of high degree and an extremely small beam divergence become necessary. This is achieved by long source-to-sample and sample-to-detector distances (of the order of several meters) and use of thick lead blocks as collimators. The beam cross-section is typically of the order of 0.2×5 mm^2 and beam divergence is in the range 5′–10′.

The goniometer is of conventional design but with a provision for holding comparatively large crystals. In structure factor measurements, crystals with linear dimensions 3–8 mm are employed and in mosaicity studies, the linear dimensions of crystals used are as large as 1–8 cm. In the latter case, the goniometer has the provision for translating the sample horizontally and vertically to facilitate scanning of different volume elements. In view of the extremely narrow range of Bragg angles, the rotation of the goniometer has to be through still smaller angular ranges. A combination of SLO–SYN step motor and digital electronics ensures rotation in steps of 2″ (and sometimes even 0.2″) around the vertical axis. Similar precision is needed in the setting and movement of the detector.

Generally, a NaI(Tl) scintillation detector is used for measuring the intensity of the directly transmitted beam and an intrinsic Ge detector for the measurement of the Bragg reflection intensity; as mentioned earlier, the latter provides a high signal-to-noise ratio eliminating the need for background correction.

Alkire and Yelon[13] have discussed the design of the γ-ray diffractometer set up at the University of Missouri Research Reactor. Hailed as a 'facility (that) marks the beginning of a new era in GRD', this is a fully automated full-circle diffractometer. The main difference between this instrument and the European instruments is the use of a 1000 Ci strong ^{153}Sm source. In view of the very short half-life (Table 1), the source requires frequent irradiation in the reactor. For this reason, a transportable source holder designed for underwater handling of the source material is required and is described by Alkire and Yelon. Because of the short half-life, intensity measurements stretch over several half-lives. The measurements made in different runs need careful normali-

Table 1. Properties of some γ-ray sources used in GRD

Source	Activity	Half-life	Wavelength (Å)	Wavelength spread ($\Delta\lambda/\lambda$)	Other relevant features
^{198}Au	80 Ci	2.7 days	0.0301 0.078	10^{-6} 10^{-6}	Very weak absorption; source has to be replaced every week; commonly employed for absolute structure factor measurements; suitable for thick samples/heavy elements
^{192}Ir	200 Ci	74 days	0.0205 0.0265 0.0392 0.0603	10^{-6} 10^{-6} 10^{-6} 10^{-6}	0.0392 Å line generally used; presence of four lines makes it useful especially in determination of extinction-free structure factors
^{153}Sm	1000 Ci	46.8 h	0.12	10^{-5}	Suitable for study of thin samples/light elements
^{137}Cs		29.9 yrs	0.020		

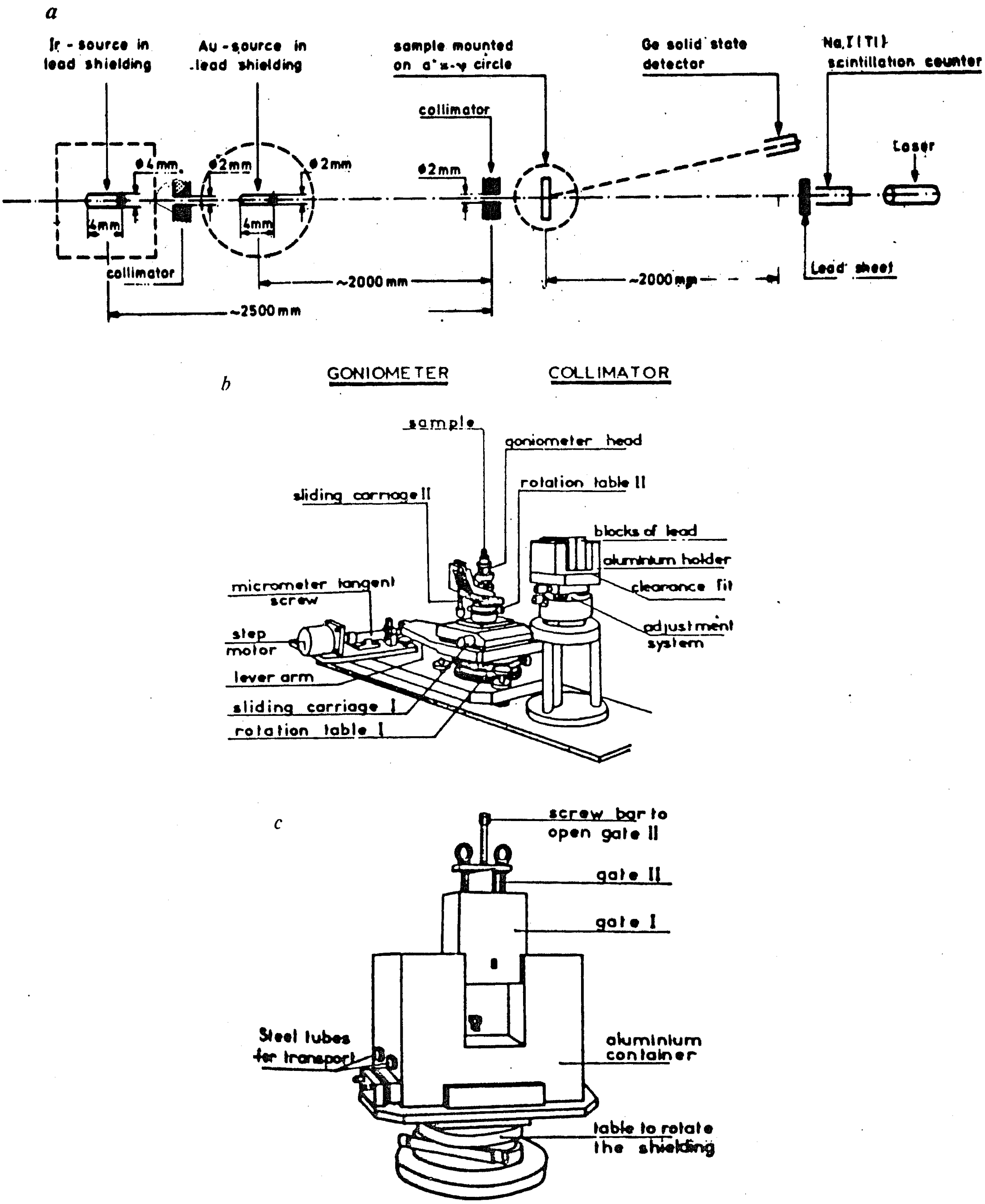

Figure 1. A typical γ-ray diffractometer (refs 11 and 12). *a*, Schematic drawing of the set-up; *b*, Goniometer; *c*, Source holder.

zation. On the other hand, the high strength of the source and the consequent intense flux make it ideal for studies on light elements and thin samples. The spectrum of the ^{153}Sm source contains several wavelengths, each monochromatic. The availability of several wavelengths makes it possible to determine extinction-free structure factors. The instrument has a linear drive for the detector with which the sample-to-detector distance can be changed to effect different resolution requirements.

The diffractometers referred above are one-crystal diffractometers; their angular resolution is about 10″. Schneider[11] states that with this angular resolution, fine structure of rocking curves cannot be resolved. Double crystal diffractometers offer a better angular resolution. The first double crystal γ-ray diffractometer was built by Tanner[14] and collaborators at the Rutherford Appleton Laboratory in UK using a ^{198}Au source but has not been put to much use beyond recording the rocking curves for Si crystals.

A double crystal γ-ray diffractometer which uses a ^{192}Ir source has been installed at the Hahn-Meitner Institute; a detailed account of this is given by Schneider and Graf[15]. The principle of the double crystal γ-ray diffractometer is shown in Figure 2. As shown, the first crystal is brought into correct diffracting position by displacing the detector at distance $\Delta x^{(1)}$. Similarly, the second crystal (sample) is oriented by displacing the detector by a distance $\Delta x^{(2)}$. The second crystal (sample) is then displaced in the scattering plane perpendicular to the direct beam by a distance Δx which is determined by the Bragg angle of the first crystal and the distance between the two crystals. With Si as the first crystal with distances shown in Figure 2, $\Delta x = 16.34$ mm. The first crystal (Si), though otherwise highly perfect, has internal strains which make its (220) planes have an effective cylindrical curvature about the [111] axis with a radius of curvature of nearly 1000 m. The diffraction of the direct beam at these curved (220) planes results in the diffracted beam being collimated to 1.4″. The first crystal thus acts like a Soller slit for the second crystal. The resultant angular resolution of such a double crystal set-up is an unprecedented 1″ and makes the double crystal diffractometer uniquely useful in certain situations; some applications will be discussed later.

Applications of γ-ray diffraction

Absolute structure factors

Using GRD, absolute extinction free structure factors of Be have been determined[19] with an accuracy of 0.5%. By comparison with theoretical model calculations, good agreement was observed with the SCF-HF-LCAO calculations. Similarly the determination of the absolute structure factors of Au by GRD[7] has made it possible to differentiate between the relativistic and non-relativistic calculations of form factors.

Anisotropy of structure factors

Using GRD, Kretschmer and Schneider[16] could accurately determine the anisotropy of structure factors of vanadium metal. Absolute structure factors were determined and the ratio F_{442}/F_{600} was determined at temperatures from 70 K to 800 K. The temperature variation of this ratio was found to agree with Willis' model[17].

γ–N synthesis

It is well known that bond distances calculated from the electron charge distribution obtained from XRD are affected by the distortion of the electron cloud by bonding effects. To separate the bonding effect, a difference synthesis is carried out between the structure factors obtained from XRD and ND (the X–N synthesis[18]). One limitation in the accuracy of X–N synthesis is that the XRD and ND experiments are carried out on entirely different samples which may have different absorption, extinction and mosaicities. In view of the negligible absorption of γ-rays and neutrons by crystals, it is possible to carry out GRD and ND experiments on the same sample. The results of γ–N synthesis obtained from data on the same sample are superior to the results of the X–N synthesis[13].

Structural and optical changes in antiferromagnetic crystals

MnF_2 is a classic example of antiferromagnetic ordering; the transition takes place at 67 K. In MnF_2, the Mn ions are in special positions and the structure can be described in terms of the c/a ratio and the F ion position parameter u.

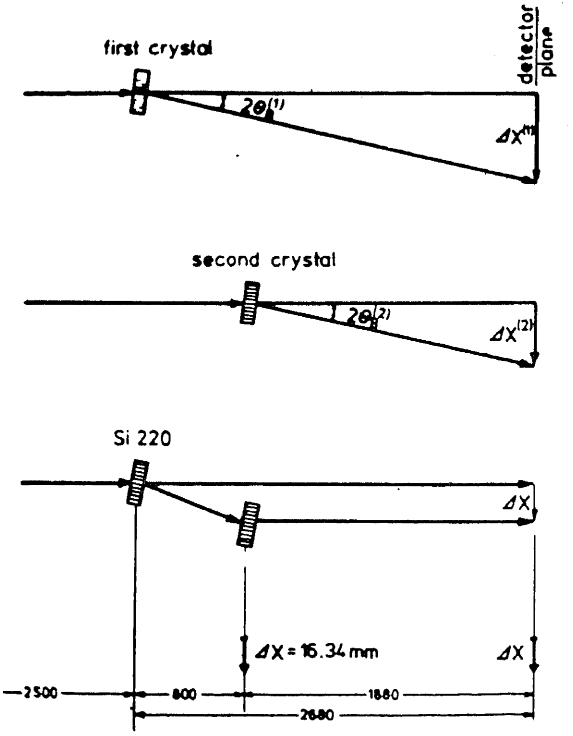

Figure 2. Schematic lay-out of a double crystal γ-ray diffractometer (ref. 14)

At the para-antiferro transition, MnF_2 shows a prominent change in the linear optical birefringence. It was found that the magnetostrictive changes in the lattice constants cannot account for the observed magnetic birefringence. However, it would be possible to account for the birefringence on the basis of the Ewald and Born theory (see ref. 19) in terms of a small (fifth place) magnetostrictive change in the u parameter at low temperatures.

The best X-ray determination[20] of the u parameter (Table 2) gave results with fourth place accuracy which was not sufficient to test the Ewald–Born theory results. As mentioned earlier, several corrections required in processing of XRD and ND data become unnecessary in GRD due to which structural parameters can be determined with much greater accuracy. Jauch *et al.*[8] carried out a GRD determination of u at room temperature and low temperatures with fifth place accuracy. Their results are given in Table 2. The change in u obtained from their results leads to a magnetic birefringence comparable with the observed birefringence. These results have been confirmed by an independent GRD study by Jauch *et al.*[21] (Table 2). The time-of-flight neutron diffraction technique (TOFND) is capable of yielding structural data with accuracy comparable with GRD, although it does not have the several advantages that arise from the short wavelengths of γ-rays. Jauch *et al.*[21] determined the u parameter of MnF_2 using the TOFND technique. Their value for u (given in Table 2) at room temperature (RT) is comparable with the GRD value. However, their low temperature (LT) value differs from the GRD value. This is attributed to the noncoincidence of the centroid of the electronic charge distribution (obtained from GRD) and the nuclear positions (obtained from TOFND) in the antiferromagnetic state. As a result the Δu value from TOFND is less than that from GRD.

NiF_2 is another antiferromagnetic crystal isomorphous with MnF_2. Palmer and Jauch[22] carried out exhaustive GRD studies on NiF_2 at RT and LT (in the antiferromagnetic state). Their main results are: (i) there is a magnetostrictive shift in u (Table 2) which is similar to that in MnF_2 and is consistent with optical birefringence results, (ii) using Stewart's method[23], GRD data is used to obtain the atomic charge density (Figure 3) and (iii) there is excellent agreement between the centroids of atomic charge density obtained from GRD and nuclear positions obtained from ND[24].

Mosaicity

Mosaicity is an important aspect of the imperfect state of a crystal and an important factor in determining extinction in a crystal. GRD plays a useful role in the characterization of mosaicity in crystals. The input for such studies is the determination of rocking curves, which are obtained by a step-wise rotation of the crystal through angle ω and recording the counts at every step. The special advantages of GRD in these studies are as follows.

a) In a diffraction experiment, the full-width at half maximum (FWHM) of the rocking curve ($\Delta\theta$) contains contributions from the lattice strain ($\Delta\tau/\tau$) as well as mosaicity (η). The relation[6] is

$$(\Delta\theta)^2 = (\Delta\tau/\tau)^2 \tan^2\theta_B + \eta^2 , \tag{1}$$

where θ_B is the Bragg angle. In XRD, the first term is comparable with the second whereas in GRD, because of the very small Bragg angles, the first term is negligible and, thus, the FWHM of the γ-ray rocking curve is almost entirely due to mosaicity. GRD is therefore far superior to XRD as a tool for characterization of mosaicity.

b) In order to show dynamical diffraction effects, a defect-free crystal has to have a minimum size. A measure of this size is the extinction length t_{ext} given by[12,25]

$$t_{ext} = V/(r_0 F \lambda) , \tag{2}$$

where V is the volume of the unit cell, F the structure factor, λ the wavelength and r_0 the classical electron radius. Due to the relative magnitudes of

Table 2. Values of the fluorine position parameter u in MnF_2 and NiF_2 at room temperature and low temperature

		u			Δu
Crystal	Method	Room temp.	11 K	15 K	u(RT) − u(LT)
MnF_2	XRD (ref 20)	0.3050 (2)			
	GRD (ref. 8)	0.30523 (7)	0.30471 (6)		52×10^{-5}
	GRD (ref. 21)	0.30492 (8)		0.30443 (8)	49×10^{-5}
	ND (ref. 21)	0.30491 (3)		0.30480 (2)	11×10^{-5}
NiF_2	GRD (ref. 22)	0.30364 (9)		0.30326 (8)	38×10^{-5}
	ND (ref. 22)	0.30365 (4)		0.30332 (4)	43×10^{-5}

the wavelengths of X-rays and γ-rays, t_{ext} will be much larger for γ-rays than for X-rays. Thus for a crystal of thickness t_0, $t_0 \geq t_{ext}$ for X-rays but $t_0 \ll t_{ext}$ for γ-rays. Hence, a crystal which appears perfect when studied with $\lambda = 1.54$ Å behaves like an imperfect crystal when studied with $\lambda = 0.03$ Å (ref. 25). Thus certain aspects of mosaic state can be explored only with GRD.

c) As mentioned earlier, because of the negligible absorption of γ-rays, the mosaicity of large single crystals can be studied without cutting or surface preparation. Figure 4 shows different volume elements of a niobium crystal, the corresponding rocking curves and the variation of the half-width from one volume element to another[12]. Although the integrated intensity shows some variation from one volume element to another, the FWHM and, hence, the mosaicity is essentially homogeneous.

As mentioned earlier, the double crystal γ-ray diffractometer is more powerful in giving details of the mosaicity because of the high angular resolution. The following examples are from ref. 15. Figure 5 shows the rocking curves for a Cu crystal obtained with such an instrument; it can be seen that the rocking curves are devoid of any substructure. On the other hand, the rocking curves for a Nb crystal shown in Figure 6 show considerable substructure. It may be noted that this is the same crystal for which a one crystal γ-ray diffractometer has shown rocking curves without any substructure and without much variation (Figure 4). The rocking curves for a synthetic FeS_2 crystal (Figure 7) show a larger substructure for the (111) and than for the (220) reflection planes. This indicates that the crystal growth parallel to (111) lattice planes was perturbed. Figure 8 shows the double crystal rocking curve of a [111] n-type Si wafer. An analysis of the broadened rocking curve indicates a curvature in the (111) planes with a radius of curvature of 460 m.

While the ^{198}Au and ^{192}Ir sources are useful in determining rocking curves of thick samples, only the ^{153}Sm source can handle thin samples. Figure 9 shows the rocking curves of two less than 500 µm thick samples of HgI_2 used as X-ray and γ-ray detectors. These were determined with the help of the Sm based γ-ray diffractometer[13]. It is seen that the mosaic structure of the sample marked grade D is more marked than that of the grade B sample. Also, the efficiency of the grade B sample as a detector was found to be better than that of the grade D sample. Thus, it is possible to link up detector performance with mosaic structure and use GRD in quality-control of radiation detectors.

GRD has been used to characterize the mosaicity of several other crystals like Al, Ge, Be, ice, CaF_2 and gypsum.

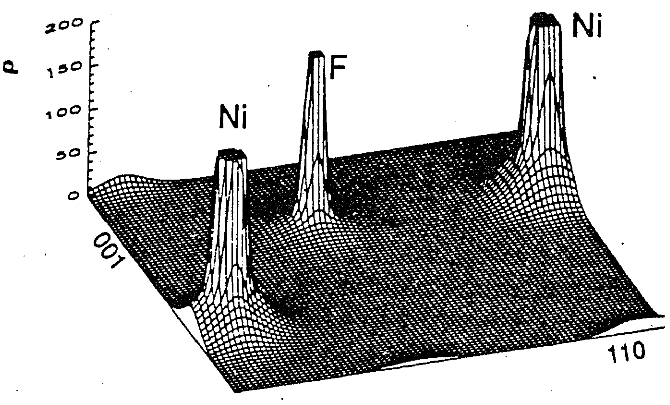

Figure 3. Total charge-density in the (110) plane of NiF_2 at room temperature (ref. 22); ρ in units of e Å^{-3}.

Domain structure

The double crystal diffractometer with ^{192}Ir was used by Palmer and Jauch[26] to study the domain wall orientation in magnetically ordered NiF_2 which has a Neel temperature of 73 K. GRD experiments were conducted at 15 K. The rocking curves for the (110), (1$\bar{1}$0) and (002) reflections have FWHM of 12″, 16″ and 22″ at RT. At 15 K, the rocking curves for the (110) and (002) reflections broaden (FWHM 38″ and 33″ respectively) whereas the (1$\bar{1}$0) rocking curve splits into two components (Figure 10). Palmer and Jauch[26] have interpreted this observation by proposing a zig-zag domain structure for NiF_2 (Figure 11).

Extinction models

It has been stated earlier that extinction is very much less in GRD than in XRD and the slight amount of extinction, if present, can be corrected in a comparatively simple manner. The ^{192}Ir source emits four wavelengths from 0.0205 to 0.0603 Å. The Bragg intensities measured at these wavelengths may be extrapolated to $\lambda = 0$ to obtain extinction-free kinematical structure factors irrespective of the nature of the extinction process. On the other hand, by assuming different extinction models, detailed corrections can be applied to these intensities and the models can be tested. Schneider *et al.*[27] considered the problem of primary extinction by studying the thickness dependence of the intensity of γ-ray diffracted by Si crystals with thickness between 1 and 3 cm and examined the Darwin model combined with the assumptions of Lorentzian and Gaussian mosaic distributions. They showed that the primary extinction can be accounted for by the sum of two Gaussian distributions. Palmer and Jauch[28] determined GRD intensities for a NiF_2

crystal using all the four wavelengths of the ^{192}Ir source. Extinction corrections were applied using different models. By a comparative analysis, it is pointed out that for treating secondary extinction, the Zachariasen[4] model and the Becker and Coppens' model[29] are much better than Sabine's[30] model.

Crystal growth studies

The determination of the rate of crystal growth in melt grown crystals is difficult as the liquid–solid interface cannot be observed externally due to the opaqueness of the containers and the surrounding furnace. According

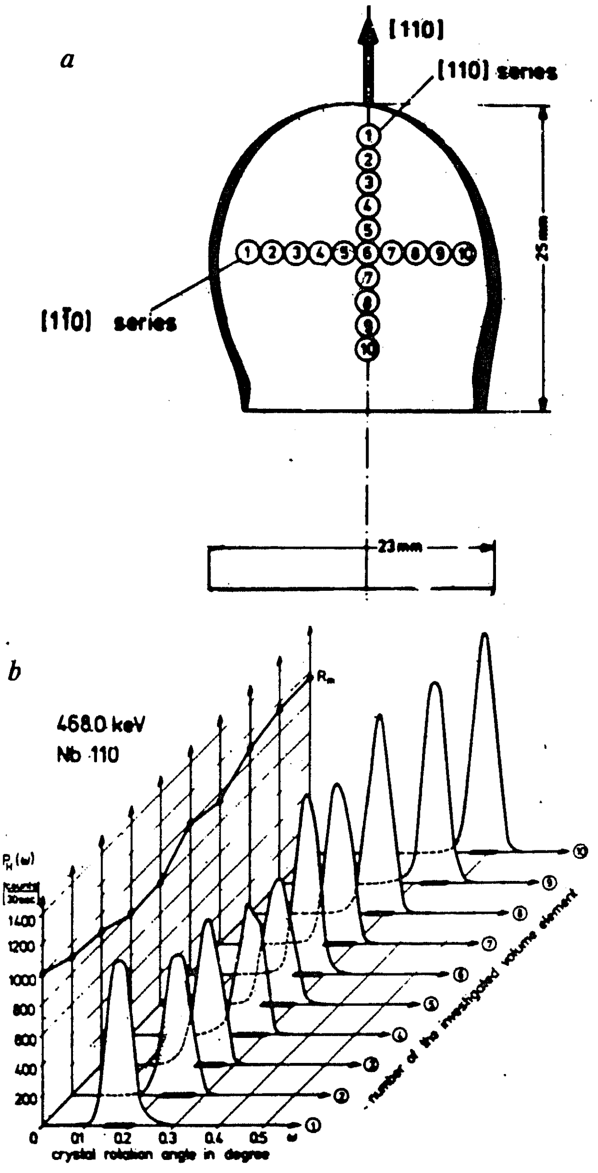

Figure 4. γ-ray rocking curves for a Nb crystal (ref. 12). *a*, Geometry and orientation of the investigated Nb crystal; *b*, Series of rocking curves measured at the (110), reflection of the Nb crystal shown in Figure 4 *a*; $P_H(\omega)$ is the Bragg reflected intensity, R_m the integrated intensity and FWHM is shown at the base of each rocking curve.

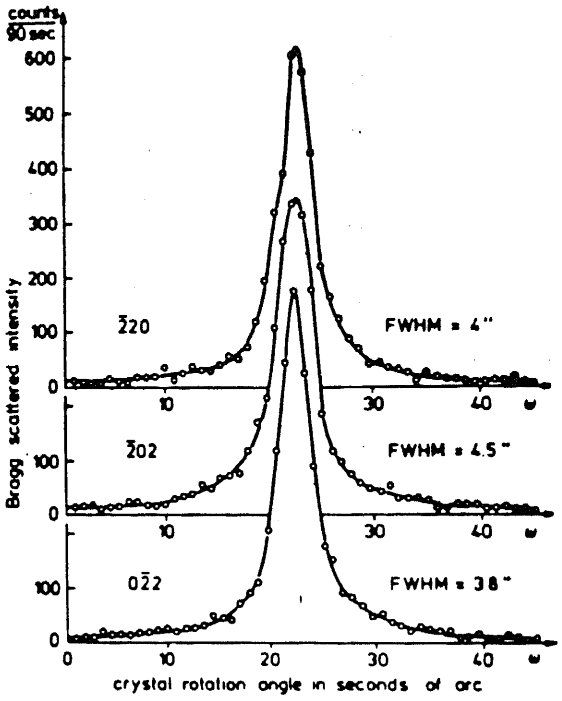

Figure 5. (220) double crystal rocking curves for a Cu single crystal.

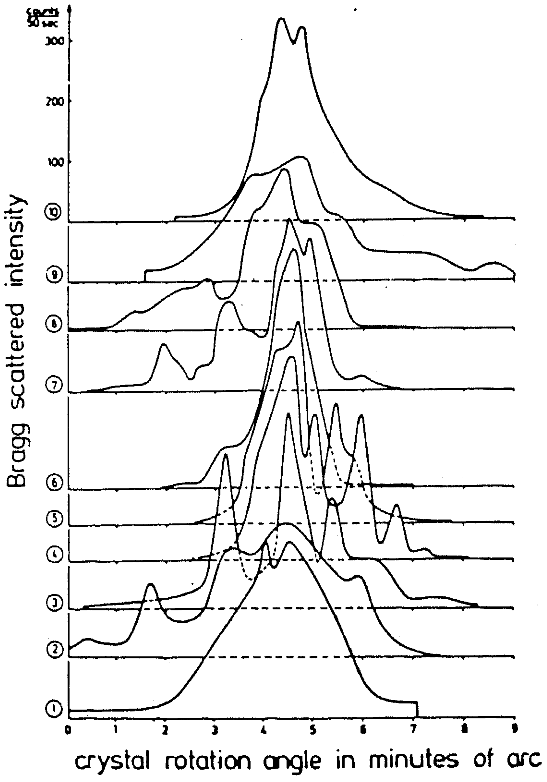

Figure 6. Double crystal rocking curves for the (110) reflection for a Nb crystal (the crystal and the numbering of volume elements are the same as in Figure 4 *a*).

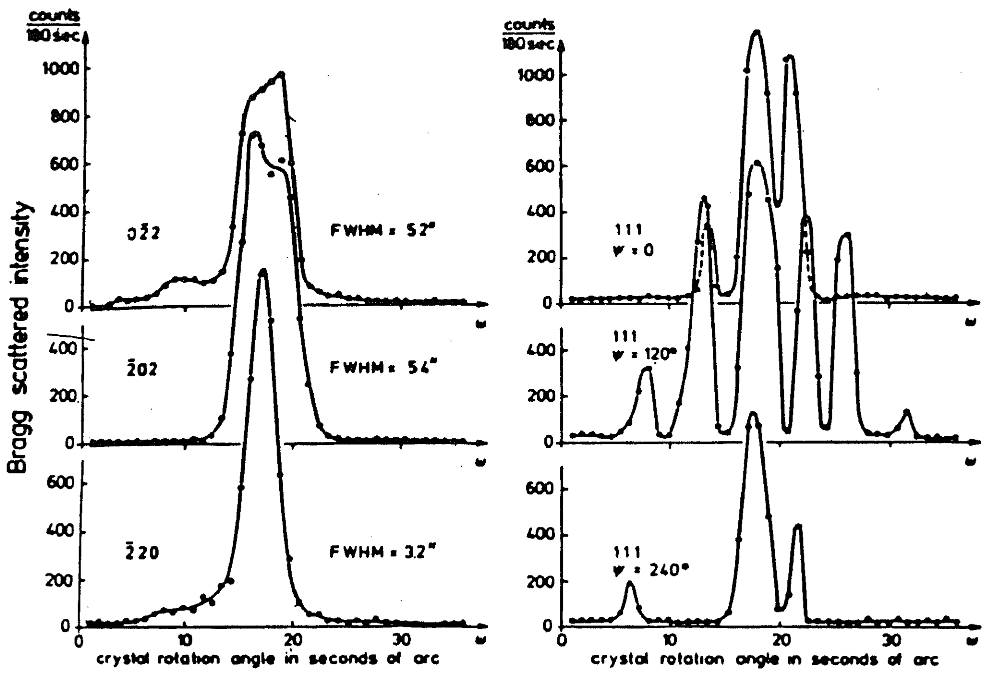

Figure 7. Double crystal rocking curves measured for the (220) and (110) reflections of a vapour grown FeS_2 crystal.

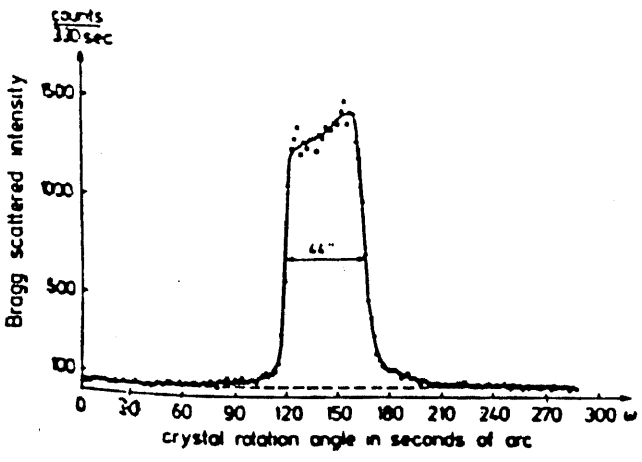

Figure 8. (111) double crystal γ-ray rocking curve of a [111] Si wafer.

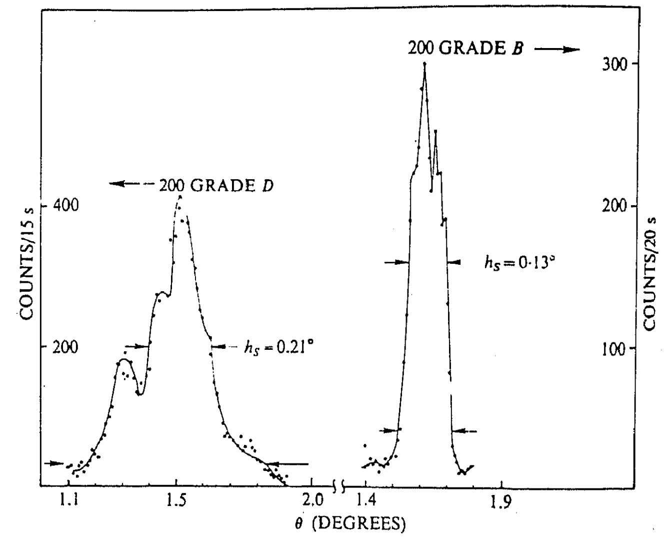

Figure 9. Rocking curves of two thin HgI_2 crystals cleaved from a single crystal (ref. 13).

to the kinematic theory of diffraction, the integrated intensity is proportional to the diffracting volume. Welter *et al.*[31] made use of this aspect and the negligible absorption of materials for γ-rays, to determine the rate of growth of a Cu crystal growing in a graphite crucible by means of GRD (Figure 12*a*). The intensity of diffraction from the solidified crystal was determined as a function of time (Figure 12*b*). As the liquid–solid interface moved up, the intensity increased, finally becoming constant as the interface swept the entire beam; the beam height was 10 mm. In this particular experiment, the growth rate was found to be 2.3×10^{-4} cm/s.

Other applications

GRD has been employed in the study of several other interesting problems like the ferroelectric-to-ferroelastic transition in KDP and RbDP (refs 30, 33), the Jahn-Teller effect in $TmSO_4$ and $TbVO_4$ (refs 34, 35), twinning in KDP[36] and pressure-induced structural changes in α-NbD_x (ref. 37).

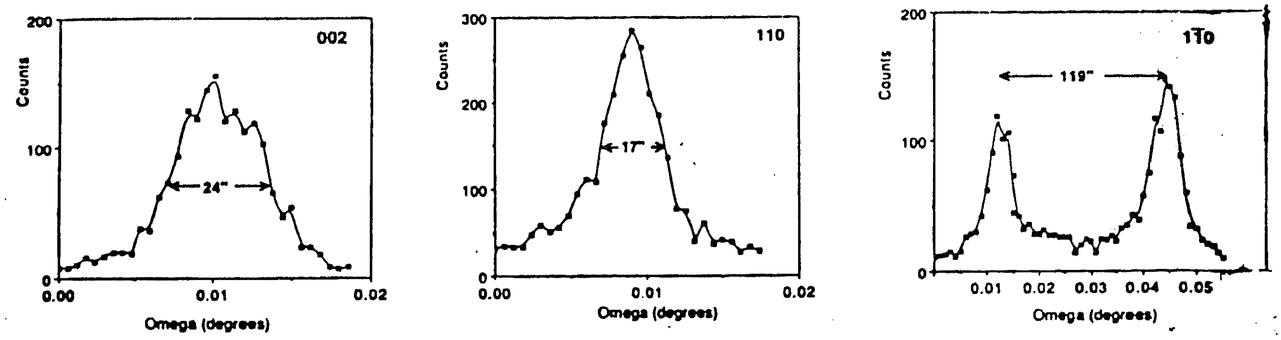

Figure 10. Rocking curves for the (002), (110), (1$\bar{1}$0) reflections of a NiF_2 crystal at 15 K and in the presence of a magnetic field along [100] (ref. 26).

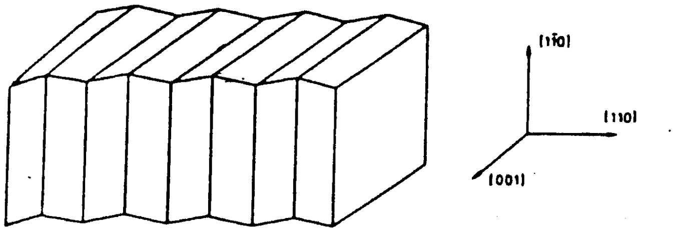

Figure 11. Zig-zag domain structure proposed for the NiF_2 crystal (ref. 26).

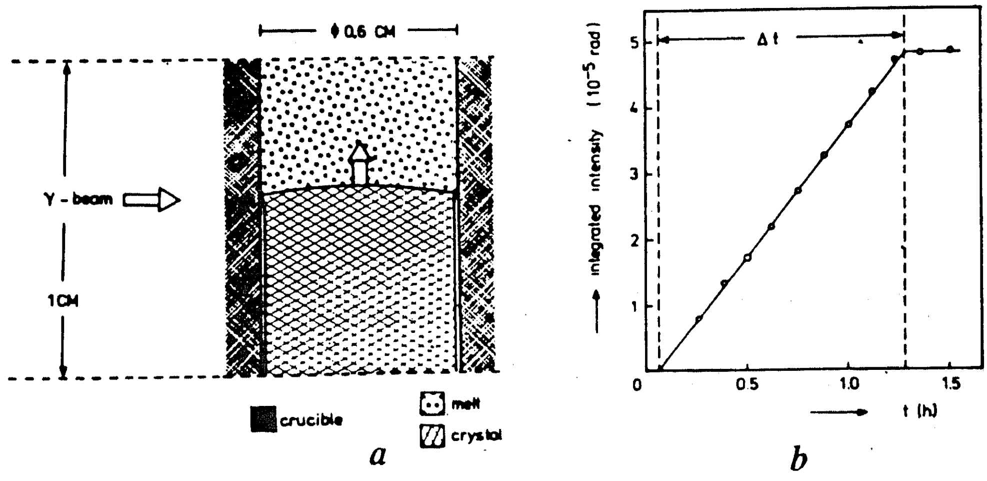

Figure 12. GRD experiment to study crystal growth rate (ref. 31). *a*, Schematic set-up; *b*, Time-dependence of the integrated intensity of the (111) reflection of the growing Cu crystal.

Conclusion

The principles, special features, experimental details and some applications of γ-ray diffraction are discussed. It is shown that the short wavelength bestows on γ-ray diffraction many advantages which do not obtain in X-ray and neutron diffraction, making it a powerful tool in structural and defect studies.

At present, γ-ray diffractometers are available at the Hahn-Meitner Institute at Berlin (Germany), the Laue-Langevin Institute at Grenoble (France), the Rutherford Appleton Laboratory (UK), the University of Missouri, Columbia (USA) and Kernforschungsanlage Julich (Germany).

More than a dozen laboratories in India have made notable contributions in the field of X-ray crystallography. Defect characterization of crystals by XRD is being carried out at NPL. The contributions of the BARC group in the field of neutron diffraction are internationally recognized. However, GRD facilities are not available in India. It is hoped that gamma-ray diffraction facility will become available in the near future, in view of its superiority in certain respects over XRD and ND.

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RESEARCH ARTICLE

Evaluation of water quality index of the river Cauvery and its tributaries

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Water quality of the river Cauvery and its tributaries – Arkavathi and Vrishabhavathi – was assessed by using Bhargava's water quality index. Significant seasonal variation was revealed by the spatially measured physico-chemical characteristics. The water quality index is categorized as class III (satisfactory range) for the Cauvery and Arkavathi rivers and class IV (poor range) for the Vrishabhavathi. The spatial homogeneity of quality was determined by using Duncan's multiple range test.

THE Cauvery river system, revered as the Ganges of Southern India, is of great economic importance as apart from being the main source of drinking water, it is utilized for industrial and agricultural purposes. It has been subjected to rapid deterioration over the years due to increasing pollution stress from various sources. Cauvery's sub-tributary Vrishabhavathi receives most of the urban wastes generated in Bangalore City before joining the river Arkavathi, which finally joins Cauvery.

Physico-chemical and biological studies on the river Cauvery have been undertaken[1–4]. However, rivers Arkavathi and Vrishabhavathi have received little attention.

Several reports on river water quality assessment using physico-chemical and biological indices have been published[5-10]. Until now the river Cauvery has not been subjected to water quality evaluation using the physico-chemical indices. In this article we evaluate the water quality using Bhargava's index[5] and determine the spatial changes in the water quality.

Study area

The river Cauvery traverses 800 km through Karnataka, Kerala and Tamil Nadu flowing from west to the east before emptying into the Bay of Bengal. It originates at Talacauvery in the Brahmagiri range of Western Ghats in Coorg district of Karnataka at an altitude of 1341 m above MSL. It has a drainage area of 81,155 sq km with a mean annual flow of 20,950 m^3 and storage capacity of 5.478 million m^3. It receives agricultural, domestic and industrial wastes. The sampling stations selected on this river are situated between 77°18′25″ to 77°27′5″ longitude and 12°6′50″ to 12°18′5″ latitude.

The river Arkavathi, a tributary of Cauvery has its source near a tank in the south western region of Nandi Hills (Karnataka) at an elevation of 1480 m. It has an average flow of 850 m^3. It takes a south westerly course passing through Bangalore rural district for 190 km before confluencing with Cauvery at Kanakapura. The discharges are usually from industries, sewers and agricultural fields. The sampling stations are situated between 77°22′10″ to 77°24′10″ longitude and 12°18′30″ to 12°36′10″ latitude.

The river Vrishabhavathi, a sub-tributary of Cauvery is known to originate in Bangalore city and traverses through the city before joining river Arkavathi near Muduvadidurga. Seventynine industries in Bangalore discharge effluents into it, of which 21 are major and 58 are small scale industries. Fiftytwo per cent of the major and 7% of the small industries have treatment plants. The river also receives copious amount of domestic waste from Bangalore city. The sampling stations are situated between 77°25′30″ to 77°26′12″ and 12°46′30″ to 12°47′30″ longitude and latitude respectively.

Description of sampling stations

Station 1. Muththathi, an upstream station of Cauvery situated 120 km from Bangalore is a recreation spot. The flow of water is rapid and annual fairs are conducted near the Bheemeswari temple with the water being used for bathing and washing.

Station 2. This station is about 18 km from Bangalore near Shanumangala. The flow of water is slow and it is occasionally brownish. Water is used for irrigating fodder crops and vegetables.

Station 3. It is 2½ km downstream station 2 near Hanchipura. The low level of water results in stagnancy during summer and it becomes brownish occasionally. Water is used to irrigate vegetable plots.

Station 4. It represents a point on the outlet of the Byramangala tank situated 4 km downstream Hanchipura. The water is usually green or bluish green due to recurrence of algal blooms. *Eichhornia crassipes* L. is abundantly found along the entire stretch of the tank. The water is used for irrigating several crops (Figure 1).

Station 5. It is situated 48 km from Bangalore near

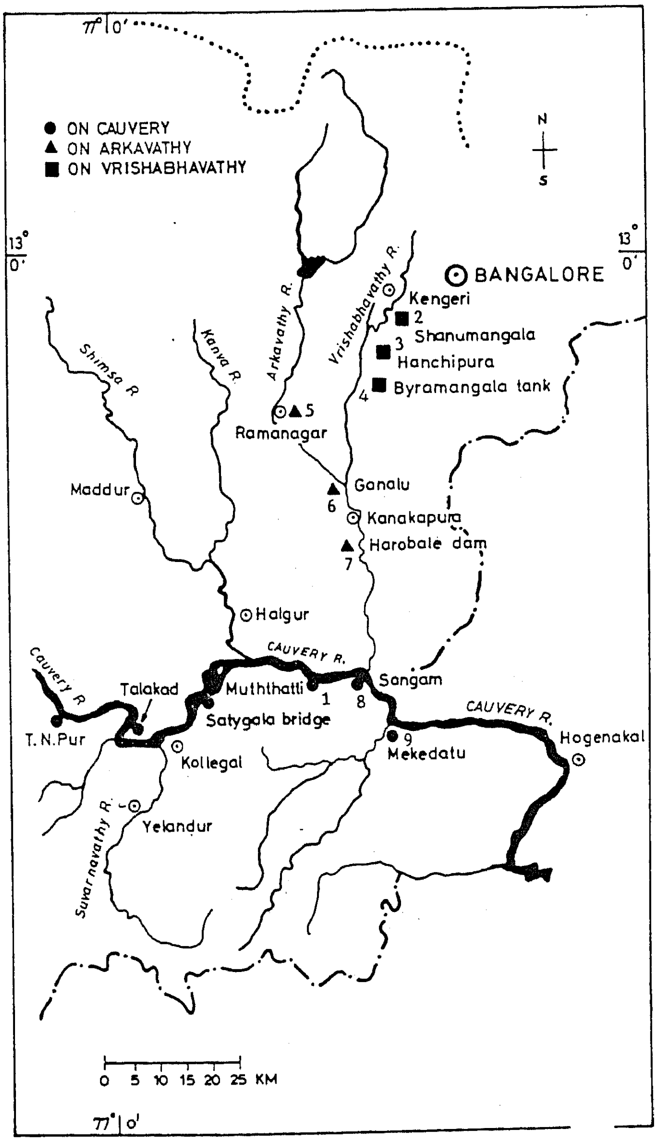

Figure 1. Map showing the locations of sampling stations.

Ramnagar. This point remained completely dry for three months (February 1991 to April 1991) during the study period. Sufficient rainfall increased the level of water during the remaining period of study.

Station 6. This is the point after the confluence of Arkavathi and Vrishabhavathi near the village Ganalu, about 8 km down station 5. This point also remained dry from February 1991 to April 1991. The water is used for bathing, washing animals and clothes.

Station 7. Located near Harobale, situated about 26 km from station 6, is the site of the construction of a dam (Harobale dam). The dam was under construction at the beginning of the study and nearing completion at the end of sampling period.

Station 8. It is situated 16 km from Muththathi. This place is called Sangama and it represents the confluence point of the tributaries (Arkavathi and Vrishabhavathi) with Cauvery. It is a famous tourist spot with water being used for washing and non-contact recreation.

Station 9. It is located 6 km down station 8. The width of the river gets reduced as it passes through a gorge, namely Mekedatu, the goat's leap. This region is undisturbed except on occasions (Figure 1).

The various stations on Cauvery and its tributaries are given in Table 1.

Material and methods

Water samples were collected in jerricans (5 l capacity) with well-fitted caps for a period of two years from August 1990 to July 1992. Samples for estimating dissolved oxygen (DO) and biochemical oxygen demand (BOD) were collected separately in polythene bottles. Water temperature was recorded on the spot. The samples were transported to the laboratory in ice boxes and standard methods of preservation were used[11].

The parameters estimated and the methods used are given in Table 2.

Results

The surface water temperature recorded lower ranges in winter while the higher ranges were encountered either during monsoon or summer. In Cauvery, the temperature ranged from 21.5 to 31.5°C, in Arkavathi from 22.5 to 31.5°C and in Vrishabhavathi from 22 to 29.25°C (Table 3).

Electrical conductivity exhibited higher ranges (0.74–2.10 mmhos/cm) at Vrishabhavathi and lower ranges (0.28–1.44 mmhos/cm) at Arkavathi. However, the annual averages for conductance were lowest in Cauvery. The conductivity decreased with dilution following rains. However, during summer the concentrate effect brought about by evaporation of water and culminating in the accumulation of salts produced a reverse trend among the stations of Vrishabhavathi.

The stations on Cauvery, namely Muththathi (131.95 mg/l), Sangama (161.22 mg/l), Mekedatu (145.89 mg/l) along with those of Vrishabhavathi (Shanumangala 239.45 mg/l) and Arkavathi (Ramnagar – 170.56 mg/l) exhibited higher winter values of total hardness. However, Hanchipura and Byramangala (300.83 and 259.33 mg/l respectively) showed higher summer averages. Ganalu and Harobale on Arkavathi registered higher monsoon averages (255.06 and 220.44 mg/l). Nonetheless low monsoon averages were evident in Cauvery and Vrishabhavathi while Arkavathi was characterized by low summer values.

The principal pollution indicator, DO showed marked seasonal variations. All stations of Cauvery and a few on the tributaries (1.56–6.52 mg/l) showed lower summer averages. However, Hanchipura exhibited lower monsoon (0.74 mg/l) and Ganalu registered lower winter (5.81 mg/l) average. Further, Byramangala, Ramnagar, Ganalu and Harobale on the tributaries along with Sangama and Mekedatu which together form a continuous stretch, exhibited higher monsoon mean.

BOD was minimum in winter and maximum in summer at all the stations of Cauvery and ranged from 0.13 to

Table 1. Stations on Cauvery and its tributaries

Station	River
Muththathi	Cauvery
Shanumangala	Vrishabhavathi
Hanchipura	Vrishabhavathi
Byramangala tank	Vrishabhavathi
Ramnagar	Arkavathi
Ganalu	Point after the confluence of Arkavathi and Vrishabhavathi
Harobale	Arkavathi and Vrishabhavathi
Sangama	Cauvery
Mekedatu	Cauvery

Table 2. Parameters and methods used for the analysis of water samples

Parameter	Method
Temperature	Direct measurement[11], p. 126.
Conductivity	Systronics conductivity meter, Model 304 (ref. 11), pp. 76–80.
Dissolved oxygen	Modified Winkler's method[11], pp. 418–419
Biochemical oxygen demand	Modified Winkler's method[11], pp. 525–532.
Chlorides	Argentometric method[11], pp. 287–288
Total hardness	EDTA titrimetric method[11], pp. 210–214
Ammoniacal nitrogen	Preliminary distillation step[11], pp. 379–380 Nesselerization method[11], pp. 377–379

22.67 mg/l. Ramnagar and Harobale recorded 3.61 and 5.75 mg/l of BOD during summer while during winter the average demand was 1.94 and 1.55 mg/l. Ganalu recorded lower summer (1.51 mg/l) and higher winter (3.97 mg/l) average. In Vrishabhavathi, Shanumangala registered lower monsoon (14.86 mg/l) and higher summer (21.19 mg/l) averages whereas Hanchipura and Byramangala exhibited high (21.68 and 20.32 mg/l) winter averages.

Chlorides, the chemical component indicative of pollution of animal origin[12], exhibited variable seasonal trends among stations of Cauvery. On Arkavathi higher concentrations were noted during monsoon (95.88–153.88 mg/l) while low summer (47.42 and 114.88 mg/l) values were seen at Ramnagar and Harobale.

Ammoniacal nitrogen, another indicator of pollution of animal origin, is toxic to fish in higher quantities. At Sangama and Mekedatu, lower summer (0.12–0.13 mg/l) and higher monsoon (0.52–0.69 mg/l) ranges were observed while at Muththathi, the minima (0.09 mg/l) was during winter. Ganalu and Harobale on Arkavathi (0.03–1.28 mg/l) and all the stations of Vrishabhavathi (0.38–4.87 mg/l) showed variations similar to those of Sangama and Mekedatu. Ramnagar showed lower summer (0.19 mg/l) and higher winter (1.49 mg/l) averages.

The use of water was grouped into five categories as suggested by Bhargava[5] with different water quality parameters for each beneficial use. The factors were given weightage based on its relevance to any particular use. The water quality index (WQI) was determined according to Bhargava's method[5].

The simplified model for WQI for a beneficial use is given by

$$\mathrm{WQI} = [n^{n}_{i=1}\, fi\,(Pi)]^{1/n} \times 100\,,$$

where n is the number of variables considered more relevant to the use and $fi\,(Pi)$ is the sensitivity function of the ith variable which includes the effect of weighting of the ith variable in the use.

To evaluate the overall WQI at a point where the river is simultaneously used for several beneficial uses, the weight of each beneficial use is taken into consideration. The extent of change in the sensitivity function value would depend on the variable's degree of relevance to the use (Table 4).

On stations of Cauvery, hardness and temperature contributed to deterioration in water quality for some of the beneficial uses. At stations of Arkavathi, chlorides along with the hardness and temperature decreased the quality of water considerably. DO, BOD and ammoniacal nitrogen affected the water quality significantly in Vrishabhavathi.

At several stations, the overall water quality decreased during summer and improved during monsoon. The overall water quality index was placed under class II for Cauvery, class III for Arkavathi and class IV for Vrishabhavathi. In Vrishabhavathi for several uses, the quality fell under class IV or V. Ramnagar of Arkavathi is grouped under class II or III while the other stations come under class III or IV for most uses and class V for agricultural use. At Muththathi, the index was comparatively higher than Sangama and Mekedatu, being classified under class I or class II for varied uses (Table 5).

The analysis of variance and the results of the Duncan's multiple range test helped in grouping the stations on the basis of water quality. Multiple comparison of means was made to indicate significant differences in the means of water quality index. This test divides the means into subgroups such that any two means in a subgroup do not differ significantly. Accordingly, Shanumangala and Hanchipura formed one subgroup, Ganalu and Harobale the second subgroup and the stations of Cauvery, the third subgroup. Byramangala and Ramnagar were unique in their water quality and differed from all other subgroups.

Discussion

The fluctuations in water temperature followed the trend in atmospheric temperature. The higher ranges during monsoon were due to various factors like turbidity and non-return of monsoon[13]. Turbid streams are known to have a greater heating capacity than clear ones[14]. Further, turbidity interferes with deeper penetration of light whereby surface water gets heated up.

The conductance of water gives an estimate of the quantity of ionizable chemical substances and also the trophic status. The trophic status is in turn determined by the density of planktons. Accordingly, the trophic status varies from oligotrophic condition in clear water to polysaprobic nature in respect of polluted streams. The seasonal fluctuations in conductance were dependent on water discharge. Lower conductance during monsoon and highest in summer in the study on Ganga, Yamuna and Tons is reported[15].

The categorization of hardness[16] suggested that it ranged from moderately hard to very hard in Vrishabhavathi. However, the means during different seasons indicated very hard range (> 250 mg/l) except at Hanchipura, where during monsoon hardness decreased to hard range (200–250 mg/l). In Arkavathi, it varied from slightly hard (100–150 mg/l) to very hard range. In Cauvery, it ranged from soft (< 50 mg/l) to moderately hard (150–200 mg/l) range.

DO determines the quality and quantity of biota[15]. At Harobale, DO showed drastic fall (0.95 mg/l in March) and rise (4.19 mg/l in April) during summer which

Table 3. Range values of some physico-chemical parameters (mg/l) at different stations

Parameter	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6	Station 7	Station 8	Station 9
Temperature (°C)	21.50–31.00	22.50–28.50	22.00–29.25	22.50–28.50	22.50–28.50	23.50–30.50	25.00–31.50	24.00–31.50	23.00–31.50
Electrical conductivity (mmhos/cm)	0.22–0.43	0.79–1.87	0.78–2.10	0.74–1.67	0.28–1.25	0.47–1.44	0.30–1.30	0.21–1.17	0.11–0.80
Total hardness	60.66–146.66	172.66–350.66	174.00–358.66	151.00–337.34	72.00–328.67	120.66–329.34	125.34–306.32	48.00–212.68	42.66–193.00
Dissolved oxygen	5.67–7.96	0.00–7.96	0.00–8.77	1.08–7.29	0.00–7.43	4.12–7.56	0.95–8.36	5.81–7.56	5.67–7.83
Biochemical oxygen demand	0.13–4.87	4.88–64.80	7.02–36.40	3.24–30.60	0.40–15.36	0.27–15.36	0.20–22.67	0.17–5.94	0.13–4.46
Chlorides	12.31–28.87	91.83–222.47	91.35–216.79	81.41–215.84	15.62–226.25	38.34–221.05	26.51–202.59	12.31–154.31	12.30–98.93
Ammoniacal nitrogen	0.01–1.96	0.02–8.75	0.01–13.34	0.01–6.31	0.01–8.65	0.02–2.03	0.01–3.10	0.02–2.10	0.01–1.06

Table 4. Suggested values of parameters for the various classes of the beneficial use

Uses	Sensitivity function	Relevant parameter values					
		Temp (°C)	DO (mg/l)	BOD (mg/l)	Chlorides (mg/l)	Hardness (mg/l)	NH_3 nitrogen (mg/l)
Bathing, swimming	1.0	–	> 8.0	< 1.0	–	–	0.0
	0.8	–	5.8	3.0	–	–	0.6
	0.5	–	4.0	6.5	–	–	1.5
	0.2	–	2.5	10.0	–	–	2.4
	0.01	–	< 2.0	> 12.0	–	–	> 3.0
Public water supplies	1.0	–	> 8.0	< 1.0	–	–	–
	0.8	–	5.8	3.0	–	–	–
	0.5	–	4.0	6.5	–	–	–
	0.2	–	2.5	10.0	–	–	–
	0.01	–	< 2.0	> 12.5	–	–	–
Agriculture	1.0	–	–	–	< 4.0	–	–
	0.8	–	–	–	20.0	–	–
	0.5	–	–	–	50.0	–	–
	0.2	–	–	–	80.0	–	–
	0.01	–	–	–	> 100.0	–	–
Industry	1.0	–	–	–	–	< 20	–
	0.8	–	–	–	–	100	–
	0.5	–	–	–	–	200	–
	0.2	–	–	–	–	320	–
	0.01	–	–	–	–	> 400	–
Non-contact recreation	1.0	15	> 8.0	< 1.0	< 20.0	–	–
	0.8	20	6.0	3.5	100.0	–	–
	0.5	28	4.2	8.0	250.0	–	–
	0.2	35	3.0	12.0	400.0	–	–
	0.01	> 40	< 2.5	> 15.0	> 500.0	–	–

Table 5. Computed seasonal values of Bhargava's water quality index for the study period

	For beneficial uses																	
	Bathing, swimming			Public water supply			Agriculture			Industrial			Non-recreation			Overall WQI		
Station	M	W	S	M	W	S	M	W	S	M	W	S	M	W	S	M	W	S
1	85†	95*	88†	96*	96*	89†	92*	90*	80†	89†	85†	86†	81†	85†	76†	89†	90*	84†
2	6**	10**	4**	16§	11§	4**	10**	10**	10**	62‡	54‡	57‡	15§	13§	10**	22§	20§	17§
3	4**	15§	5**	10**	16§	5**	10**	10**	10**	59‡	53‡	51‡	15§	22§	8**	20§	23§	19§
4	11§	12§	17§	23§	14§	18§	10**	10**	10**	67†	61‡	57‡	37‡	23§	21§	30§	24§	25§
5	77†	57‡	57‡	84†	77†	59‡	51‡	50‡	49‡	76†	77†	81†	75†	73†	58‡	73†	67†	61‡
6	72†	75†	89†	82†	71†	80†	10**	25§	10**	59‡	61‡	61‡	73†	68†	73†	59‡	60‡	63‡
7	70†	70†	66†	86†	88†	63‡	10**	26§	10**	65†	69†	74†	73†	66†	57‡	61‡	60‡	54‡
8	85†	92*	87†	93*	92*	88†	75†	62‡	82†	85†	80†	84†	80†	81†	75†	84†	84†	83†
9	88†	92*	86†	94*	93*	87†	78†	84†	84†	86†	82†	85†	80†	80†	70†	85†	86†	82†

Rating:			
	*Class I	(Excellent)	> 90
	†Class II	(Good)	65–89
	‡Class III	(Satisfactory)	35–64
	§Class IV	(Poor)	11–34
	**Class V	(Acceptable)	< 10.

M, Monsoon; W, Winter; S, Summer.

coincided with significant increase and decrease in algal density. Higher monsoon averages at several stations are perhaps due to greater turbulence[17]. Vrishabhavathi registered nil DO during several months as a result of enormous input of organic wastes. In this case DO and BOD were below and above the prescribed standard limits respectively[18].

Chlorides were the second major anions after bicarbonates. Mitra[19] reported similar trend in Mahanadi and attributed the variations in the concentration of chloride to differences in the flow. Comparison of the background concentration of chlorides in Arkavathi (Ramnagar) showed an increase by 33% after the confluence of Vrishabhavathi with Arkavathi near Ganalu. A significant correlation between chlorides and ammoniacal nitrogen (0.475 and 0.455 at $P > 0.05$) was observed at Ramnagar and Harobale.

The water quality of Cauvery is in the excellent to good range mainly due to lower input of industrial and/or municipal wastes. The polluted waters of the tributaries are diluted to a great extent by Cauvery as the inflow from them is comparatively less and variable. Further, the observed values of DO, BOD and ammoniacal nitrogen both during monsoon and summer indicated optimum self-purification occurring in the system. Therefore, it can be said that the present built-in purifying capacity of Cauvery is sufficient to tolerate the existing pollution load. In general the quality oscillated between poor to satisfactory range for Arkavathi after confluence with Vrishabhavathi, while before it tends to remain in good to satisfactory range (Ramnagar). Vrishabhavathi water was of poor or unacceptable quality because of the enormous amount of urban wastes discharged into it.

The uniqueness of water quality of Byramangala is mainly due to its morphometry, being situated on the outlet of a tank. The tank acts as a sink facilitating sedimentation of most components. As a consequence, in contrast to Shanumangala and Hanchipura of Vrishabhavathi, the outlet point at Byramangala showed lower hardness, chlorides and conductance. Similarly, Ramnagar differed from Ganalu and Harobale as it is the point before the confluence of the tributaries.

Therefore, it is inferred that Vrishabhavathi brings about considerable lowering of the water quality of Arkavathi but had no significant impact on Cauvery. The Duncan's test helped in strengthening the conclusion drawn by the use of water quality index. A comparison of DO and BOD values indicated that Cauvery is not largely affected by the inflow from the tributaries while water quality of Arkavathi deteriorates considerably after its confluence with Vrishabhavathi.

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A neuro-expert system model for conflict resolution

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We describe here a neural network and expert system model for conflict resolution of unconstrained handwritten numerals. The neural network classifier is a combination of modified self-organizing map and learning vector quantization. The basic recognizer is the neural network. It solves most of the cases, but fails in certain confusing cases. The expert system, the second recognizer, resolves the confusions generated by the neural network. The results obtained from this two-tier architecture are compared with those of a combination of four algorithms. This work shows that it is possible to eliminate the substitution while maintaining a fairly high recognition.

NOTWITHSTANDING the progress accomplished in the last few years in the field of pattern recognition, the problem of matching human performance in handwritten character recognition remains a challenge even today[1]. Most of the recognition accuracy rates reported so far are over 95% (ref. 2). While these systems have no difficulty with well-formed samples, their challenge is to maintain a high performance level with samples which are distorted or written in more 'personal' styles. Furthermore, since errors are costly and delay service, maximum reliability is required.

Based on the consensus of individual opinions observed in human interaction, the Concorde University research team has developed a multiple expert system (4 algorithms approach)[3]. The performance of this system was compared with the opinions given by a group of human experts. Ideally it is required that the system must be able to differentiate the unrecognizable and confusion categories and to give the same counts as those given by human experts. Also, in case of confusions the machine should produce the same confusing pair as human experts do. The multiple expert system does not fulfill these expectations. It is suggested that a statistical and/or neural network model would be a good complement for the multiple expert system[3]. Here we present a neuro-expert system for unconstrained handwritten numeral recognition. Interest in neural networks is rapidly growing and several neural network models have been proposed for various difficult problems, especially classification problems[4]. Traditional classifiers test the competing hypothesis sequentially, whereas neural network classifiers test the competing hypothesis in parallel, thus providing high computational rates[5,6].

Expert system is a validation module which makes a more informed decision about the classification than the neural network. We have proposed a generalized artificial neural network-based expert system tool for character recognition, especially in case of confusions. The confidence levels obtained from the neural network are stored in the knowledge base using frame model of knowledge representation[5].

The issue taken up here is to resolve the confusion between the conflicting numerals by eliminating the substitution error, and to compare the results of the developed system with the results of the combination of four algorithms method.

The conventional feature extractor converts input handwritten characters into a highly compressed form[6]. This method is suitable only for the uppercase letters of the English alphabet. However, the feature extraction of all the unconstrained handwritten characters which are written in different styles is not possible. For example the horizontal features extracted for the samples using this method are not recognized properly. This disadvantage is taken care of in the modified feature extractor by making the vertical regions unsymmetrical.

The modified encoding method makes use of a 15 × 15 bit map as shown in Figure 1. The figure shows (i) three horizontal regions (H_1, H_2, H_3), (ii) six vertical regions (V_1, V_2, V_3, V_4, V_5, V_6) and (iii) four diagonal regions (D_1, D_2, D_3, D_4). Using these thirteen regions, thirteen features of the pattern are extracted. These features are used during the training process of the neural network. The training process is carried out using Kohonen's modified self-organizing map (MSOM) and learning vector quantization method (LVQ).

Kohonen's self-organizing map uses unsupervised learning to modify the internal state of the network and

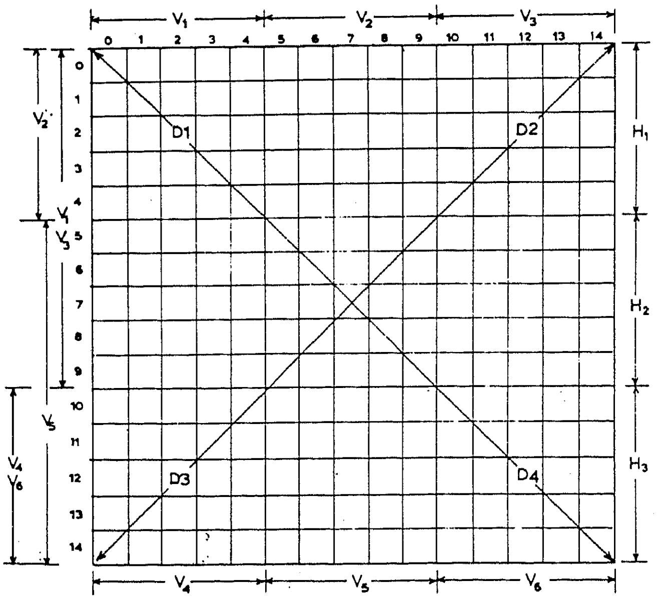

Figure 1. Thirteen-segment encoder.

to model the features found in the training data set. The map is autonomously organized by a cyclic process of comparing the input patterns to the vectors at each node. The node vector with which the inputs match is selectively optimized to present an average of the training data. Then all the training data are represented by the node vectors of the map. Thus starting with a randomly organized set of nodes, the proposed method proceeds to the creation of a feature map representing the prototypes of the input patterns.

In the conventional SOM the misclassification is more[4,9] because the minimum distance formula is applied for both the standard (machine printed numerals) and the distorted sets of samples. This is overcome in MSOM by applying the minimum distance formula for only the standard set of samples. A sample is compared only with the nodes represented by the standard set of samples (which form the centroids for each class). Using the minimum distance formula and the winner node (that is the node which matches the sample most), the weights in the neighbourhood of this winner node are updated. The MSOM is organized by a cyclic process of comparing the input patterns to vectors at each node twice. The so obtained MSOM is further fine-tuned using the LVQ technique[9,10].

Kohonen has suggested that if the nodes of the SOM are used for pattern recognition, their classification accuracy can be multiplied if nodes are fine-tuned using supervised learning principle[9].

The LVQ uses supervised learning to modify the internal state of the network provided by MSOM and to remodel the features found in the training data. A fine-tuned map is autonomously organized by a cyclic process of comparing input patterns to the vectors at each node. Fine-tuning is achieved by selecting training vectors **X** with known classification, and presenting them to the network to examine the cases of misclassification. The best match comparison is performed at each node and the winner node is noted. By means of the LVQ algorithm, patterns are self-organized into a fine-tuned feature map.

We have introduced a two-tier architecture-character recognition system (Figure 2). The basic recognizer is the neural network, which recognizes most of the cases correctly but fails in certain confusing cases and expert system is the second recognizer, which resolves the confusion generated by the neural network.

The confidence levels obtained from the neural network are stored in the knowledge base using the frame model of knowledge representation[5]. This knowledge is captured and applied to the validation module (expert system) for conflict resolution. The output from the neural network is double checked by the validation module whose inference engine decides if the sample could be a potential confusion. If there is no confusion, the output from the validation module is the same as the neural network output with a higher confidence level. If there is a high probability of confusion, the inference engine calls the appropriate specialized module which will give

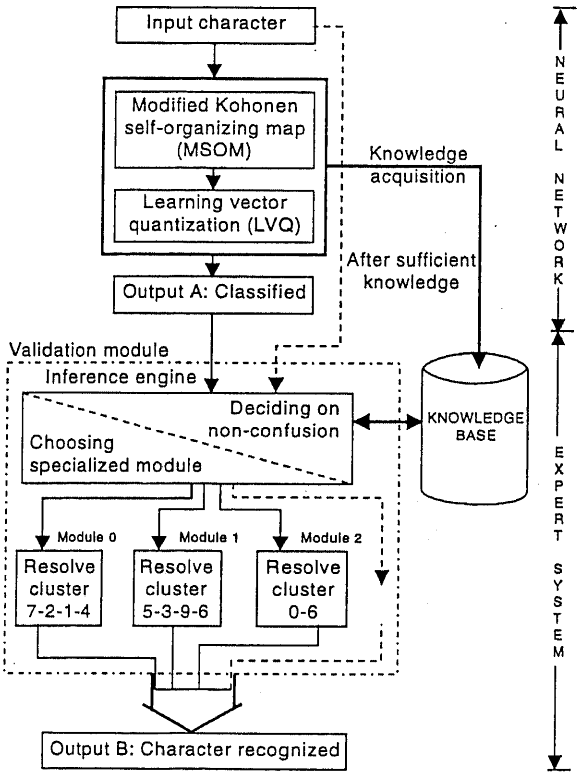

Figure 2. Two-tier architecture of the neural network expert system.

Table 1. Performance comparison of 4 algorithms approach and neuro-expert system

					Confusion in case of human experts						
		Samples rejected by human experts			Conf.					Unambiguous	
Type of system	No. of samples	Rec. (%)	Conf. (%)	Rej. (%)	Rec. (%)	Same pair (%)	One member same (%)	Different pair (%)	Rej. (%)	Sub. (%)	Rec. (%)
4 Alg. method	360	33.33	33.33	33.33	50	35	55	10	0	11	89
Neuro-expert system	360	87.5	0	12.50	69	44.4	44.4	11.11	0	0	100

the final output that is different from the neural network output. In the above process the average experts' opinion is computed. The pattern with maximum average opinion (maximum confidence level) is recognized. The knowledge modelling stage needs to be executed only once for a training set of patterns. But the decision-making stage will be executed for every test pattern.

The operations carried out by the expert system are as follows: (i) Mapping of conflicting character onto the classifier and collecting the confidence levels. (ii) Searching for the maximum confidence level. (iii) Searching for the next maximum confidence level. (iv) Comparing the maximum and the next maximum confidence levels for checking the presence of confusion. (v) In case of a confusion, define the clusters which contain one or more interrelated classes. (vi) Processing of the current confidence levels of a set of samples into their equivalent knowledge representation form. (vii) Growth of the knowledge base on the basis of current history. (viii) Finding the cluster that contains maximum confidence value class. (ix) Collecting the opinions of experts about the classes of a cluster. (x) Finally, computing the average experts' opinion for each class. The class with maximum experts' opinion contains the recognized character.

The sample database for the experiment has been generated by taking samples selected from various papers of Concorde University (which were originally selected from 17,000 sample database of US Postal service collected from various parts of USA) as seed values.

We have chosen the net size as 15×15 in which the initial neighbourhood size is 9, i.e $N_j(0) = 9$ and the number of input nodes (features) is 13. The value of alpha at various stages during the training process is as follows.

No. of iterations	Alpha
0 to i	0.923971
i to $2i$	0.4120
$2i$ to $3i$	0.23420
$3i$ to $4i$	0.051246
$4i$ to $5i$	0.0018231

where $i = (2 * \text{no of samples} * N_j(0))$ DIV 5, i.e. the total number of iterations.

Table 1 shows a comparative performance of the 4 algorithms approach[2] and the neuro-expert system. The set of 360 samples (most difficult to recognize) which were classified by a group of human experts[2] was presented to the neuro-expert system and the results obtained were compared with those of the 4 algorithms approach. The following observations were made.

(i) The samples rejected by human experts were presented to the two systems, the neuro-expert system uniquely recognized (Rec.) 87.5% of the samples as compared to 33.33% of the 4 algorithms approach.

(ii) The samples that were considered to be confusing by human experts were presented to the two systems, the neuro-expert system recognized 69% as compared to 50% of the 4 algorithms approach. Out of the remaining the 4 algorithm approach gives confusion pairs of which 35% of the samples correspond to the

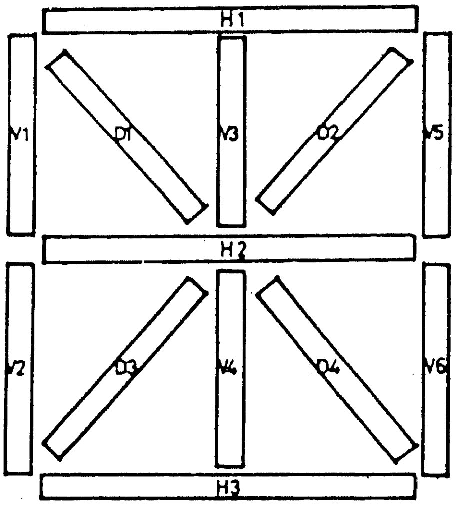

Figure 3. Conventional bar mask encoder.

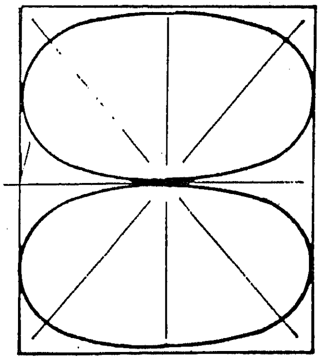

Figure 4. Machine-printed numeral 8.

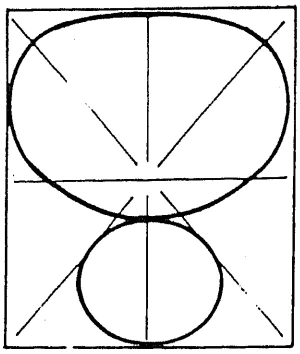

Figure 5. Unconstrained handwritten numeral 8 with horizontal feature displaced downwards.

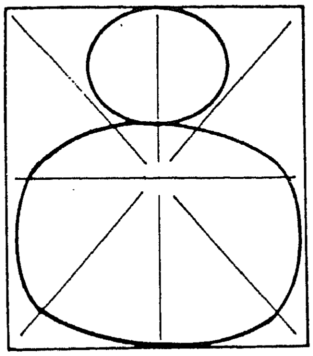

Figure 6. Unconstrained handwritten numeral 8 with horizontal feature displaced upwards.

same pairs as those given by the human experts. For 55% of samples it gives a pair in which one digit member of the confusion pair is common in both and it gives a totally different pair for the remaining 10% of samples. The neuro-expert system gives confusion pairs of which 44.4% of the samples correspond to the same pairs as those given by the human experts. For 44.4% of samples it gives a pair in which one digit member is the same as that in the human pair and it gives totally different pair for the remaining 11.11% of samples.

(iii) Of the samples which were uniquely classified by human experts, 89% were recognized by 4 algorithms approach and there was substitution in case of 11% of the samples. The neuro-expert system recognized all the samples and the substitution (Sub.) rate is nil.

From the above observations we can infer that the performance of the neuro-expert system is comparable with that of the 4 algorithms approach.

The better performance of the neuro-expert system can be attributed to the following:

1. *Choice of features.* The feature extraction method (Figure 3) has been modified to suit the recognition of unconstrained handwritten numerals. The vertical regions have been made unsymmetrical to cope with the displaced horizontal features. The above point is illustrated in Figures 4–6. Figure 4 shows a machine-printed numeral 8, the horizontal feature exactly lies on horizontal region H_2, but in Figures 5 and 6 the horizontal feature is displaced and lies in H_1 and H_3 respectively. Thus the horizontal feature which is most important in this case (since it distinguishes 8 from 0) is not extracted consistently. This problem occurs with many other numerals also. This drawback can be eliminated by making the vertical regions unsymmetrical and enabling them to extract the horizontal features as well along with the horizontal regions. In the modified method the displaced horizontal features will be taken care of by the vertical regions. Thus a number of conflicts will be resolved, eliminating the substitution error and increasing the recognition rate.

2. *Use of expert system.* By using the expert system as a validation module, the reliability of the system increases. The neural network classifier acts as a basic recognizer, which recognizes most of the samples correctly. Difficult or distorted samples which cannot be recognized by the neural network are passed on to the expert system, which is capable of taking a more informed decision. The neural network identifies the pair among which confusion occurs and the expert system is then used to resolve the confusion. Even the samples recognized correctly by the neural network are passed onto the expert system which checks the decision and returns a higher confidence level. This increases the reliability of the system.

In this paper a neural network using the modified Kohonen self-organizing map and learning vector quantization techniques, and expert system model for conflict resolution on ambiguous patterns has been accomplished. The results lead to infer some interesting properties of the human behaviour in the classification process. The developed system resolves the confusion completely. The paper also shows that the results obtained from the neuro-expert system using MSOM and LVQ are comparable with the 4 algorithms approach developed by the research team of Concorde University. The system is robust and accurate in the recognition of unconstrained handwritten characters. This two-tier architecture has been very useful in resolving conflicts of unconstrained handwritten characters in PIN or ZIP codes of mailing addresses. In particular, this work should be very useful in overcoming the dead letter problem of postal department.

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Preparation of GaN and InN for blue light emitting sources

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Semiconductors based on nitrides of Al, Ga and In have gained considerable interest recently for the successful implementation of bright blue and blue-green light emitting sources. Although most of the devices are based on hexagonal (wurtzite) GaN films, cubic (zinc blende) GaN films are of great interest because of possible growth on GaAs. We have prepared GaN and InN from *n*-GaAs and InAs single crystal wafers by NH_3 treatment at 700°C. X-ray diffraction measurements showed both cubic and hexagonal phases of GaN and InN. N_2 plasma treatment of liquid phase epitaxy grown InGaAs at 900°C resulted in growth of separate phases of only cubic GaN and InN. Optical absorption studies on GaN showed a direct band-gap of 3.46 eV. Photoluminescence measurements on GaN showed a defect-related emission band at 2.75 eV.

THERE is now considerable interest in wide-band gap semiconductors for applications in high temperature electronics as well as blue light-emitting sources. The latter are being eagerly sought to increase the density of storage in optical memories since a decrease in wavelength by a factor of 2 from 800 nm to 400 nm is expected to increase the storage capacity by a factor of 4 (ref. 1). Recently Isamu Akasaki[2] and Nakamura *et al.*[3–5] at Nichia, Japan have reported the operation of blue lasers with emission at 400 nm based on gallium nitride (GaN). Pulsed mode operation was first demonstrated[2], followed by cw operation for short durations.

Here we report the first work in India on the preparation of GaN and InN. This was done by treatment of GaAs and InAs single crystals with ammonia at high temperature (700°C).The grown layers were characterized by X-ray diffraction and photoluminescence measurements. Experiments on liquid phase epitaxy (LPE) grown InGaAs by N_2 plasma treatment at 900°C resulted in growth of separate phases of only cubic GaN and InN.

Nitrides are also of interest for high temperature electronics and their preparation and properties have been reviewed by Davis[6]. A programme of work on nitrides was initiated in our laboratory in the late eighties and resulted in the laser deposition of BN[7] and AlN[8] on GaAs and InP. Later the conductor TiN widely used as a diffusion barrier in Si technology was also deposited[9]. The series AlN–GaN–InN is attractive for LED and laser applications as these are all direct gap semiconductors with $E_g = 5.6$ eV, 3.4 eV and 2.07 eV respectively and exhibit complete solid solubility. SiC, on the other hand, with $E_g = 2.6$ eV has an indirect gap resulting in LEDs with much lower efficiencies. The technology of blue sources using the II–VI semiconductor ZnSe is also well developed[1], but the efficiency of GaN-based devices is expected to be higher.

We have prepared GaN and InN from *n*-GaAs and InAs single crystal wafers by NH_3 treatment in a home-made continuous flow reactor which was placed in a muffle furnace whose temperature was controlled to ± 1°C. The temperature of nitridation is critical since As evolution from GaAs and InAs is known to occur above 600°C.

Chemically cleaned GaAs (100) and InAs (100) single crystal wafers were etched and dipped in dilute HF solution. Samples were loaded into the reactor immediately after taking out from the HF solution. The reactor was then purged by nitrogen flow for one hour. Nitridation was then carried out at 700°C for 2 h under NH_3 whose flow rate was 10 cc/min. The colours of GaN and InN films were found to be golden and blackish respectively. From X-ray diffraction measurements using CuK_α radiation ($\lambda = 1.54$ Å) (Figure 1 *a*) three peaks related to GaN were observed at 32.8°, 35° and 39.5°. These peaks represent hexagonal (1010), (0002) and cubic GaN (002) respectively. It is known that under ambient conditions GaN has the wurtzite form and as such is grown on sapphire (Al_2O_3) substrates. However it can be grown with zinc blende structure on GaAs as a strained layer, due to appreciable lattice mismatch, i.e. 4.550 Å vs 5.653 Å. This is desirable for technological reasons since a conducting substrate simplifies device geometry and yields lower series resistance, a non-trivial problem with high band-gap materials.

X-ray diffraction peaks (Figure 1 *b*) at 31°, 51.2°, 60.8° and 35.6° related to InN were observed from the InN/InAs samples. These peaks represent hexagonal (0020), (1100) and (2000) and cubic (200) InN respectively. Attempts to grow GaN on GaAs using NH_3 in r.f. plasma at 250°C were not successful. Next a lattice-matched epitaxial layer of $In_{1-x}Ga_xAs$ ($x = 0.47$) grown on InP (100) by liquid phase epitaxy was taken. The layer had been fully characterized[10] and was 4.5 μm thick with $n = 6.7 \times 10^{16}$ cm^{-3}. When this was heated in a 450 kHz induction furnace at 900–950°C in a nitrogen plasma for 2 h the surface became ash coloured. The X-ray diffraction measurements of the sample showed the formation of both InN and GaN with cubic zinc-blende structures (Figure 1 *c*). The larger diffraction linewidths in Figure 1 *a* are due to smaller grain size. Use of the Scherrer formula shows that the grain size in this case is 95–190 Å compared with 420–540 Å for Figure 1 *b* and 390–514 Å for Figure 1 *c*. This may be attributed to higher bond breaking energy for GaAs compared with InAs and lower tem-

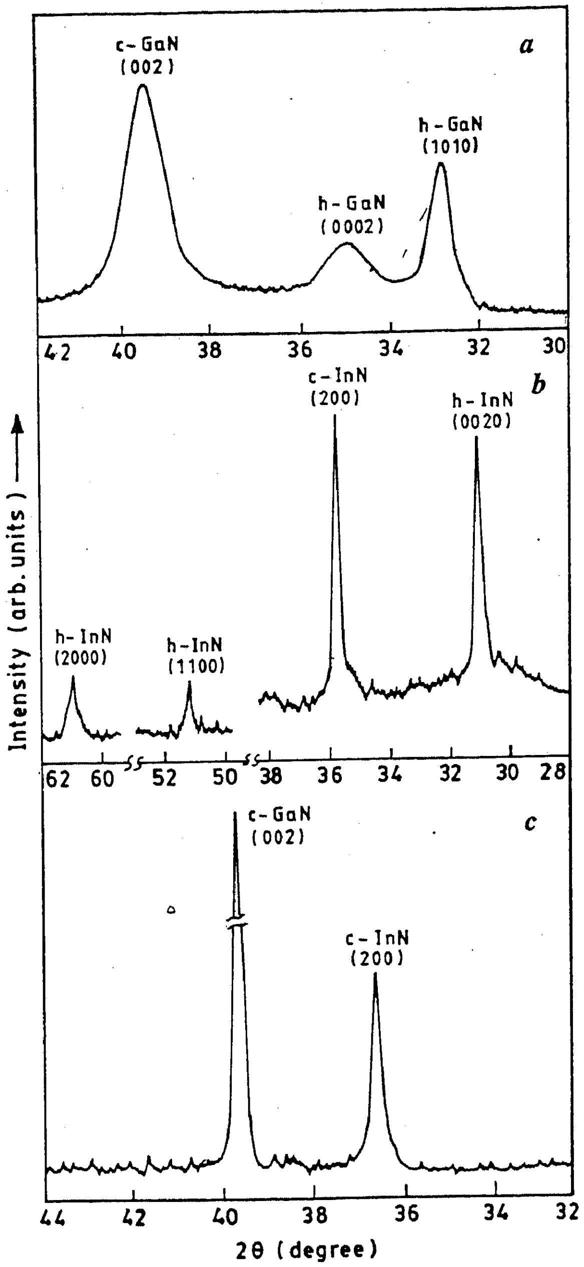

Figure 1 *a–c*. *a*, XRD scans ($\theta - 2\theta$) of GaN obtained from NH_3-treated GaAs. *b*, XRD scans ($\theta - 2\theta$) of InN obtained from NH_3-treated InAs. *c*, XRD scans ($\theta - 2\theta$) of N_2 plasma-treated InGaAs.

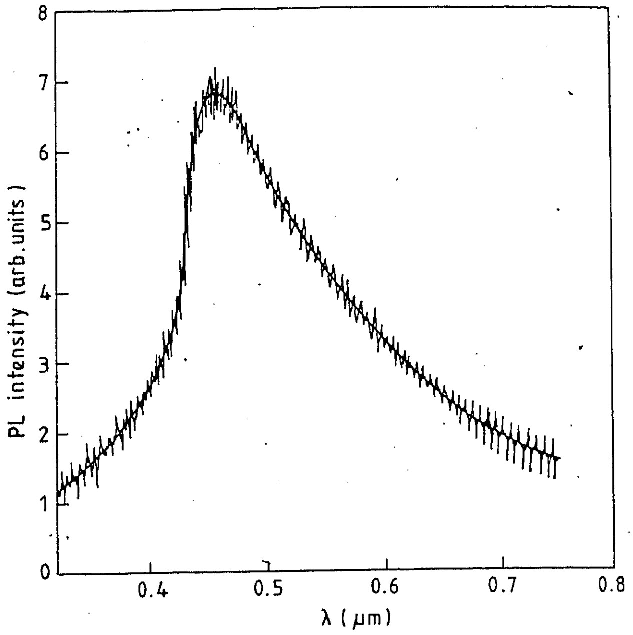

Figure 2. Photoluminescence spectrum of GaN at 300 K.

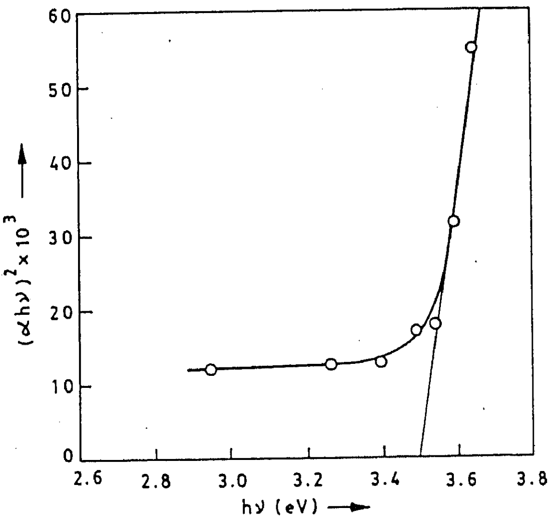

Figure 3. Optical absorption spectra of powder GaN at 300 K.

perature of nitridation, 700°C for Figure 1 *a* compared with 900–950°C for Figure 1 *a*.

Photoluminescence (PL) measurement was carried out on GaN/GaAs and InN/InAs samples at 300 K using 330 nm emission as an excitation source from a tungsten halogen lamp, Jobin-Yvon monochromator and a photomultiplier detector. Only one broad emission band (Figure 2) with peak energy 2.75 eV was obtained. A similar broad band with peak energy 2.9 eV has been observed for polycrystalline GaN films grown on Si at 400°C (ref. 11). No photoluminescence was observed from InN/InAs samples. This is intriguing since InN is considered to have a direct gap of 2.07 eV but no PL measurements have been reported from it[12]. Optical absorption measurements on powder GaN samples (Figure 3) showed a direct band-gap of 3.46 eV.

Summarizing, thermal nitridation on GaAs and InAs in NH_3 formed both cubic and hexagonal GaN and InN respectively while nitridation on InGaAs in N_2 plasma formed only cubic GaN and InN. Photoluminescence measurement on GaN showed a defect-related emission band at 2.75 eV and optical absorption showed a direct band-gap of 3.4 eV.

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Detection of the *lcr* gene in *Yersinia pestis* responsible for the recent outbreak of plague in India

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Rapid detection of plague bacilli is particularly germane to plague epidemiology since untreated bubonic plague can rapidly progress to septicaemic or pneumonic state. A 1.3 kb *Hind*-III/*Xho*-I DNA fragment from the plasmid pCd-1 which codes for the low Ca^{2+} response of *Yersiniae* spp. has been used to screen the isolates of *Y. pestis* obtained from the recent outbreak at Beed and Surat. The probe discriminated *Y. pseudotuberculosis*, diarrhoeagenic *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Shigella dysenteria* from *Y. pestis* and *Y. enterocolitica*. Both radioactive and non-radioactive probes gave excellent result.

THE once dreaded bacteria, *Y. pestis* is known to carry three plasmids of 9.5 kb, 70 kb and 95 kb size[1]. While the 9.5 kb (pPst) and 95 kb (pFra) plasmids code for the pesticine and F1 antigen respectively, the 70 kb (pCad) plasmid is responsible for the low calcium response (LCR). Expression of virulence (V-antigen) is controlled by the *lcr* gene[2].

Virulent *Yersiniae* spp. can be detected by the presence of the LCR plasmid which, besides conferring virulence to the microorganism, makes the bacteria dependent on Ca^{2+} for growth at 37°C, expression of novel outer membrane protein called *Yersinia* outer protein (Yop), increased hydrophobicity, increased affinity to crystal violet and congo red, a tendency to agglutinate in media containing mammalian serum or enhanced resistance to bactericidal effects of normal human serum[3]. Although these parameters can be used for *in vitro* detection of the microorganism, they are reported to be not reliable tests[3]. Laboratory screening requires animal passages to isolate a pure culture, phage sensitivity typing, immunofluorescent antibody against fraction-1 (a major capsular antigen), etc. Screening by these methods is expensive and time-consuming. Detection at the nucleic acid level circumvents all the above limitations and provides a rapid and sensitive method for its specific detection.

In the present study, an attempt has been made to detect the presence of *lcr* gene in *Y. pestis* by DNA probe in different human and rodent isolates obtained during the recent outbreak at Beed and Surat districts. The other two virulent non-pestis *Yersiniae*, which include *Y. pseudotuberculosis* and *Y. enterocolitica* and other diarrhoeagenic enterobacteria like *Escherichia coli*, *Klebsiella pneumoniae*, *Shigella dysenteriae* and *Salmonella typhi* were also tested both by radioactive and non-radioactive DNA probes.

The test samples used comprised of 11 clinical isolates of *Y. pestis* from pneumonic patients of Beed district and Surat city: seven isolates from rodents, *Y. pestis* strain no. A-1122 which do not carry the LCR plasmid; *Y. pseudotuberculosis* strain no. 1A which was kindly supplied by May C. Chu, WHO Collaborating Centre at Centre for Disease Control, Fort Collins, USA; the *Y. enterocolitica* strain no. 0:9 IP 383 was kindly provided by Elizabeth Carniel, WHO Collaborating Centre, Institute Pasteur, Paris. Samples of *E. coli*, *K. pneumoniae*, *S. dysenteriae* and *S. typhi*, available in DRDE, were also tested simultaneously.

Bacterial cultures (24 nos) were scraped from agar plates and each resuspended in 100 µl of 0.5 M Tris.HCl (pH 8.0), 0.1 M sodium chloride and 0.01 M ethylene diamine tetraacetic acid (EDTA) in different eppendorf tubes and lysed by lysozyme followed by heat treatment.

The 1.3 kb restriction fragment that has been used as the DNA probe, contained the 3′ end of *lcr-R*, the complete *lcr-G* and part of the *lcr-V* (virulence antigen) gene of the 70 kb LCR plasmid pCD-1. *E. coli* with the recombinant plasmid vector pES6-1, which contains the above as well as the *lcr-H*, *yop-B* and *yop-D* genes of pCD-1 was obtained from S. C. Straley, University of Kentucky, USA. A 1.3 kb *Hind*-III/*Xho*-I restricted DNA fragment from pES 6-1 was used as the DNA probe.

This DNA fragment was radiolabelled with $\alpha\,^{32}$P deoxycytidine triphosphate (dCTP) obtained from Board of Radiation and Isotope Technology, Bombay, with the help of the Klenow fragment of DNA polymerase-I (M/s Bangalore Genie Pvt Ltd) and random hexanucleotide primer (M/s Boeringer Mannheim, Germany). Non-radioactive labelling of the 1.3 kb DNA fragment was performed with digoxigenin. Both the methods of labelling were performed as per standard protocols[4].

Samples (5 µl each) were spotted on nitrocellulose membrane. A total of 24 samples were spotted in four rows and the filter was processed through the steps of denaturation, neutralization and baking.

Prehybridization and hybridization (16 h) were performed at 65°C in 6 × SSC (1 × SSC is 0.15 M sodium chloride, 0.015 M sodium citrate pH 7.0), 5 × Denhardt's solution (1 × is 0.02% each of Ficoll, polyvinyl pyrrolidone and bovine serum albumin), 0.02 M phosphate buffer, 0.5% SDS and 100 µg/ml of heat denatured sonicated Salmon sperm DNA. Post hybridization washing comprised of washing thrice in 2 × SSC and 0.5% SDS for 10 min each at room temperature followed by in 1 × SSC and 0.1% SDS for 30 min at 55°C and in 0.5 × SSC and 0.1% SDS for 30 min at 65°C.

The infrared dried filter was put for autoradiography overnight at – 20°C in case of radioactive detection. In case of non-radioactive detection, the filter was processed as advised by the manufacturer and detected by antidigoxigenin antibody conjugated with alkaline phosphatase.

Initial screening was performed with radioactive probe for 10 samples which included 3 *Y. pestis* isolated till then (isolate nos. 1, 2 and 3), *S. dysenteriae*, two isolates each of *E. coli*, *K. pneumoniae* and *S. typhi*. The 1.3 kb DNA probe detected the three *Y. pestis* (Figure 1) specifically.

Figure 2 shows the result of non-radioactive detection wherein all the 18 *Y. pestis* isolates were detected. The *Y. pestis* strain no. A-1122 which is deprived of the pCad plasmid was not detected. Although the *Y. enterocolitica* (sample no. 21) was detected as positive, the *Y. pseudotuberculosis* (sample no. 20) was not detected in repeated experimentation. Similarly *E. coli*, *K. pneumonia* and *S. typhi* (sample nos. 22, 23 and 24 respectively) were not detected.

Radioactive DNA probe used to screen all the 24 samples (Figure 3), used for further confirmation of results of non-radioactive detection, detected all the isolates which were detected by the non-radioactive DNA probe.

The samples detected as positive by the *lcr* probe has been earlier confirmed[5] to be *Y. pestis* by different classical, micro-biological, biochemical, fluorescent antibody staining and specific phage infection.

The virulence-associated low calcium response plasmid (pCd-1 also called as pCad) is shared by all the virulent spp. of *Yersinia*, viz. *Y. pestis*, *Y. enterocolitica* and *Y. pseudotuberculosis*. The LCR brings about coordinate regulation of plasmid encoded virulence genes by environmental temperature and calcium[2]. The *lcr* gene has been studied by several authors in the recent past[2–9].

The LCR plasmid varies between 70 kb (refs 10, 11) and 75 kb (ref. 6) in various spp. and strains and the Ca^{2+} dependence region spans approximately 25 kb portion[6] of the LCR plasmid. This portion of the plasmid regulates expression and secretion of anti-host proteins like LCR-V (virulence protein which is a protective antigen) and about 10 Yops. It is a regulatory response in which a set of virulence-related operons are strongly induced at 37°C in the absence of Ca^{2+} and partially down regulated at 37°C when Ca^{2+} is present in mM concentrations[5].

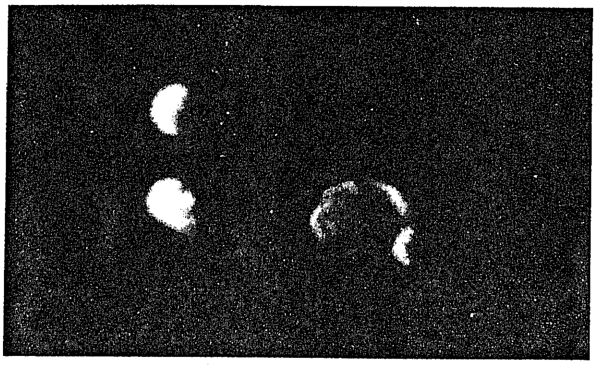

Figure 1. Autoradiograph showing three *Y. pestis* isolates harboring the LCR plasmid. The *E. coli*, *K. pneumoniae*, *S. dysenteriae* and *S. typhi* were not detected.

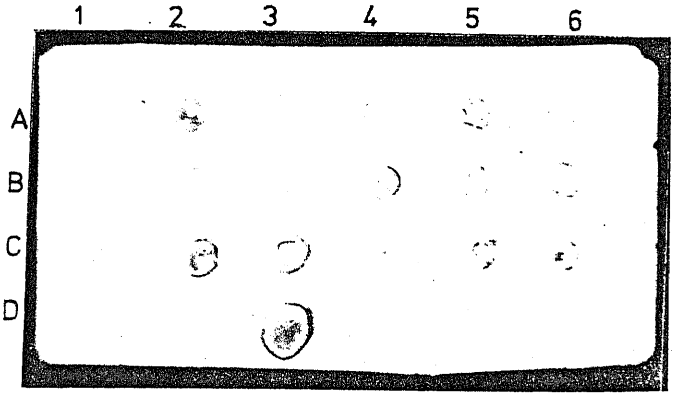

Figure 2. Digoxigenin labelled DNA probe detects all the *Y. pestis* isolates and *Y. enterocolitica* which carry the LCR plasmid. Sample nos A1 to B5 are the human isolates of *Y. pestis*, B6 to C6 are rodent isolates, D1 is *Y. pestis* A-1122, D2 is *Y. pseudotuberculosis*, D3 is *Y. enterocolitica*, D4, D5 and D6 are *E. coli*, *K. pneumoniae* and *S. typhi* respectively.

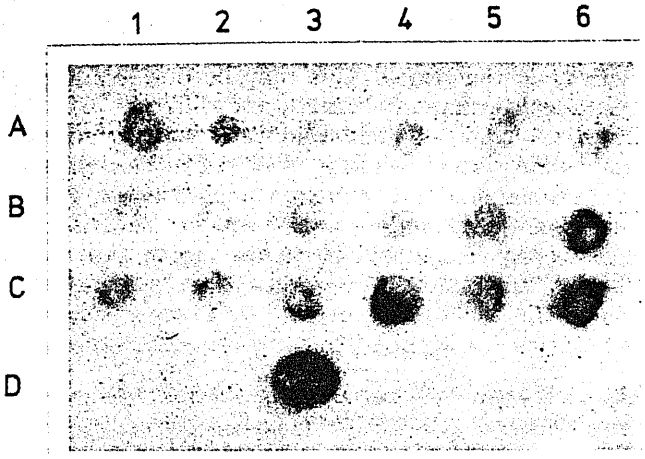

Figure 3. Radiolabelled DNA probe detects all the *Y. pestis* isolates and *Y. enterocolitica* which carry the LCR plasmid. Sample nos A1 to A6 and B2 to B6 are the human isolates of *Y. pestis*, B1 and C1 to C6 are rodent isolates, D1, D2 and D3 are *Y. pestis* A-1122, *Y. pseudotuberculosis* and *Y. enterocolitica* respectively, D4, D5 and D6 are *E. coli*, *K. pneumoniae* and *S. typhi* respectively.

All the *Y. pestis* isolates have been specifically detected by the 1.3 kb probe, excepting the strain no. A-1122 which is deprived of the LCR plasmid. Thus all the isolates of *Y. pestis* obtained from the clinical samples during the recent outbreak are harbouring the 70 kb LCR plasmid. This has also been confirmed earlier by pulse field gel electrophoresis[12]. The virulent *Y. enterocolitica*, as expected, has also been found to carry the LCR plasmid. Both the radioactive and non-radioactive DNA probes gave identical results, confirming the faint spots revealed in the non-radioactive detection, as distinct positives.

The *Y. pseudotuberculosis*, however, was not detected by the probe even on repeated experimentation. We have reduced the stringencies confining post-hybridization washings to 55°C with 0.5 × SSC and 0.1% SDS but even so there was no hybridization with the *Y. pseudotuberculosis*. The possible explanation for non-detection of *Y. pseudotuberculosis* could be (i) due to the loss or curing of the plasmid which is a common feature of the dynamic plasmid. In their studies with the 67 kb yVA-2635 plasmid of *Y. enterocolitica*, Robins-Browne[3] observed that this is a low-copy plasmid which has a high rate of spontaneous curing. They have also observed that none of the avirulent strains hybridized with the probe although they harbored the 50–70 kb plasmid. Two of the isolates detected as positive by the virulence gene probe had substantial deletion in the plasmid but still retained their virulence. They also observed that all the *Yersinia* strains carrying the LCR plasmid were virulent and that 11 of the plasmid cured strains lost their virulence. The authors therefore concluded that DNA probes consisting of the LCR-related virulence gene is an ideal test method for specific and sensitive detection of virulence of *Yersinia*. (ii) It has been reported[3] that although substantial length of the virulence plasmid is highly conserved, there exist regions of low degree homology in different spp. and strains of *Yersiniae*. It is possible that the 1.3 kb DNA fragment of the pCd-1 plasmid that we have used as the probe could be absent in the *Y. pseudotuberculosis* strain studied. Further studies with large number of strains will clarify this point.

Different other assay systems for testing virulence associated with the LCR plasmid of *Yersiniae* include Ca^{2+} dependence (which is difficult to perform, besides that it is least sensitive and specific a test of plasmid carriage), pyrazinamidase activity (also difficult to perform), crystal violet assay (a relatively better assay although it gave few false positive detections)[3]. Detection of *Y. pestis* by conventional method, besides being expensive, may take as long as two weeks' time[10]. Earlier studies by McDonough *et al.*[11] with immunodetectable antibody against the F1 antigen have proved unreliable. Chromosomal probes like invasion (inv) and attachment invasion locus (ail) have been reported to be good genus-specific probes but they cannot detect virulence *per se*[3]. The Ca^{2+} dependence region on the other hand, is a highly conserved sequence[3] and good indicator of virulence.

Detection of *Y. pestis* by polymerase chain reaction with primer pairs p1 and p2 for the 9.5 kb pPst plasmid, C1 and C2 primers for the 70 kb pCad plasmid and F1 and F2 for the 95 kb pFra plasmid have been reported[1] as an improved method. Similarly polymerase chain reaction for detection of the 501 bp *caf-I* gene fragment and a 443 bp of *pla* gene fragment from the 100 kb pFra and 10 kb pPst plasmid respectively has been reported[13] to be of useful diagnostic value.

Although recent developments including detection of *Y. pestis* through its F1 antigen with the help of a fibre optic biosensor[14] for rapid (few minutes) detection and other improved methods like polymerase chain reaction and ribotyping are available, detection of virulent *Y. pestis* by DNA probe is most promising for its cost-effectiveness, specificity, sensitivity and ability to screen a large number of samples simultaneously and inexpensively. Detection of *Y. pestis* (by a modified colony hybridization method) directly from the rat-flea[10] which transmits the plague bacilli among rats and from rats to human has been reported. The 900 bp DNA probe isolated from the 9.5 kb pKyp-1 plasmid has been reported to be suitable for plague surveillance in fleas. The *lcr* gene probe shall have to be tried for direct detection of *Y. pestis* from clinical samples as well as from infected animal tissues and carcasses. For this purpose, detection with non-radioactive system involving digoxigenin and antidigoxigenin is convenient for rapid detection in even small laboratories as rapid detection of plague bacilli is particularly germane to plague epidemiology since untreated bubonic plague rapidly progress to septicaemic or pneumonic state.

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Correlation of alpha-logger radon data with microseismicity in N-W Himalaya

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Six alpha-logger stations have been set up in the Kangra and Chamba Valleys of Himachal Pradesh (India) in N-W Himalaya to monitor radon emanation continuously. Time series radon data is recorded at all the stations from March–April 1993 to August 1995. A number of impulsive radon spikes are recorded at all the stations, most of which are correlatable with seismic activity reported by the India Meteorological Department (IMD) seismic network. All the stations manifest a unique response to seismic activity in the region. This may be due to different geological formations beneath and different diffusion rates of radon emanation at each station. Average emanation rate of radon is observed to be maximum at Jawalamukhi and minimum at Pathankot. Palampur and Jawalamukhi are found to be the most sensitive to seismic activity in the region and record the maximum number of radon spikes. Meteorological analysis of radon data revealed that radon emanation is affected up to 15% by meteorological variations.

RADON is established as a useful geochemical precursor[1-9] in earthquake prediction research since the observation of increase of anomalous radon in a deep well in Tashkent before the 1966 Tashkent earthquake[10]. The physical bases of the radon anomalies, prior to an impending earthquake, have yet to be fully understood in terms of a comprehensive theoretical model. However, it is understood that the buildup of stresses prior to a seismic event can alter the radon concentration at a position where such premonitory changes can be measured. If the earth's deformation, prior to an earthquake, releases increased amounts of radon, the temporal increase at the surface of the earth can be used to anticipate seismic activity.

The northern boundary of the Indian sub-continent, extending from the Hindukush in the west to the hills of Assam and Burma in the east, constitutes a region where the Indian plate collides with the Eurasian plate and has thus been the scene of a large number of major earthquakes in the past. During the last two decades, the N-W Himalaya has recorded five earthquakes of magnitude more than 5, viz. Kinnaur (19 January 1975), Dharamshala (14 June 1978), Dharchula–Bajang (29 July 1980), Jammu–Kathua (14 August 1980) and Dharamshala (26 April 1986). The Hindukush area and the Kangra valley of Himachal Pradesh (India) are considered to be highly seismic zones in the N-W Himalayan belt which is traversed by several major thrust faults.

Radon monitoring work was started in 1989 at Palampur in Kangra valley using the track-etch technique and emanometry. The results of the studies are reported elsewhere[8-9,11]. Under Himalayan Seismicity Project of the Department of Science and Technology (DST), Government of India, six alpha-logger probes were installed at Palampur (32.10°N, 76.51°E), Pathankot (32.30°N, 75.64°E), Kotla (32.35°N, 76.02°E), Dalhausie (32.60°N, 76.00°E), Chamba (32.55°N, 76.10°E) and Jawalamukhi (31.87°N, 76.33°E) (Figure 1). Some of these stations are situated in close proximity to the main boundary thrust (MBT) of the Himalaya. The Kangra and Chamba valleys in Himachal Pradesh are enclosed between the middle Siwaliks and the Dhauladhar range and the Pir Panjal and the Dhauladhar ranges of lesser

Himalaya, respectively. Our monitoring stations fall in the outer Himalaya and lesser Himalayan zone. Average uranium content present in the Siwalik is 3–10 ppm, much higher than the world average of 2.1 ppm (ref. 12).

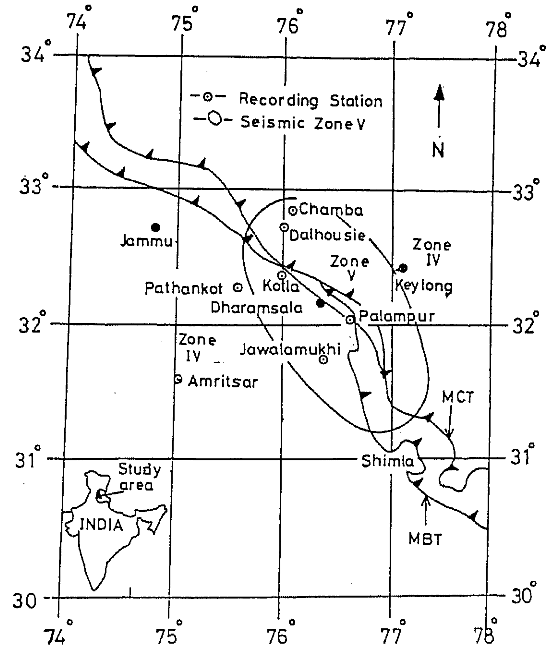

Figure 1. Radon-monitoring stations using alpha-logger probes. The major thrust faults of N-W Himalaya are demonstrated as MCT (main central thrust) and MBT (main boundary thrust). All the stations are located within high seismicity zone V, except for Pathankot which is at its periphery.

Alpha-logger probe (manufactured by Alpha-Nuclear Co, Toronto, Canada) is a portable, battery powered, microprocessor based data acquisition and control system. The unit is designed to measure near surface radon gas fluctuations over relatively short intervals of time. It consists of a silicon-diffused junction for the detection of the alpha particles and can record radon alpha counts in 15 min increments over a period of 40 days non-stop. The detector unit is placed inside a covered augur hole about 60 cm in depth. The detector is separated from the soil surface at the bottom of hole by a 6.4 cm gap, and the air in the gap shields the detector from the impact of direct alpha particles emanated by radon isotopes and their daughters. The recorded data is retrieved with the aid of a laptop IBM compatible PC. The software supplied with the system provides the facility to sum up any number of 15 min counting intervals for better counting statistics.

Except for the Jawalamukhi station, which became operational in January 1994, all the other five radon-monitoring stations became operational during March–April 1993. At some of the stations, due to severe weather conditions the battery power pack got discharged before its stipulated period of 40 days and the recorded data of a few weeks were lost. The radon data was retrieved and compiled after 4 weeks, generally.

Alpha-logger probe at Palampur station is found to record maximum number of radon spikes (Figure 2). A correlation analysis has been made to separate signal and noise from the data collected, following a specific technique. For each data set that covered a period of

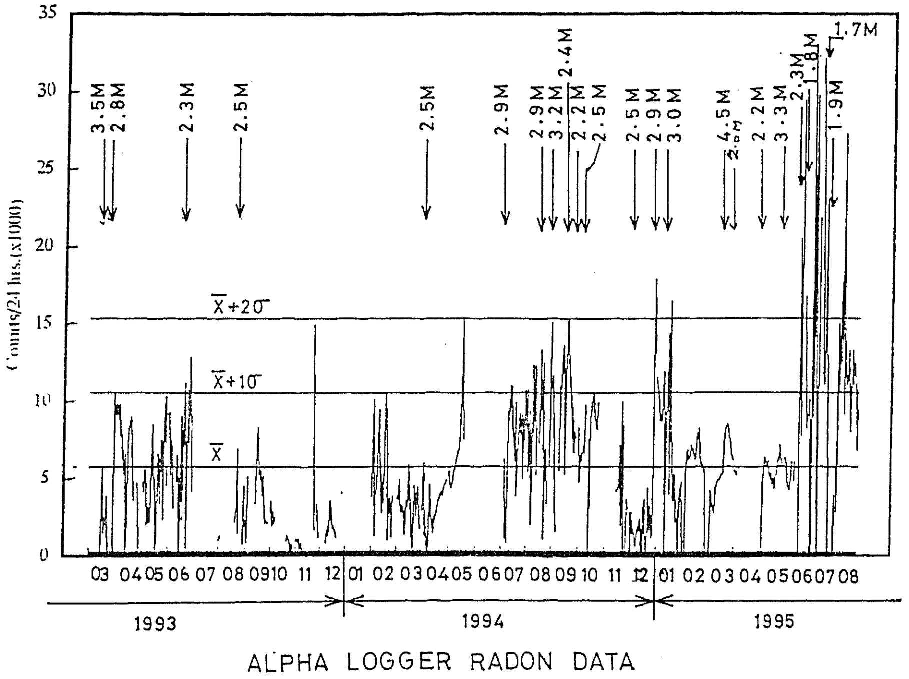

Figure 2. Alpha-logger radon data for Palampur station (arrows represent the seismic events recorded by IMD network).

Table 1. Analysis of alpha-logger radon data in soil–gas

Station	Total events (a)	Total Rn anomalies (b)	Rn anomalies preceding events (c)	Rn anomalies not preceding events (d)	Events not preceding anomalies (e)
Palampur	22	16	10	06	12
Jawalamukhi	23	20	10	10	13
Kotla	13	08	06	02	07
Dalhausie	14	10	04	06	10
Chamba	14	12	08	04	06
Pathankot	12	11	07	04	05

Table 2. Confidence level (sensitivity) of various radon-monitoring stations

Station	Signal% (c/a)	Noise% (d/b)	Confidence level (signal/noise)
Palampur	45.4	38.0	1.20
Jawalamukhi	43.5	50.0	0.87
Kotla	46.1	25.0	1.84
Dalhausie	29.0	60.0	0.48
Chamba	57.0	34.0	1.70
Pathankot	60.0	36.0	1.70

Table 3. Events correlated with radon anomalies occurring at Palampur

Date of Rn anomaly	Date of event	Lat°N	Long°E	Depth (km)	M
17–03–93	22–03–93	32.04	76.06	15.0	3.5
23–03–93	23–03–93	32.41	76.35	10.5	2.8
21–06–93	24–06–93	32.86	76.02	06.0	2.3
23–08–93	28–08–93	32.54	76.76	15.0	2.5
02–04–94	10–04–94	31.55	76.90	15.0	2.5
05–07–94	07–07–94	31.42	76.90	15.0	2.9
19–08–94	19–08–94	31.72	76.63	19.0	2.9
31–08–94	08–09–94	32.54	76.43	15.0	3.2
20–09–94	25–09–94	32.23	76.47	15.0	2.4
28–09–94	02–10–94	32.64	75.86	57.0	2.2
09–10–94	15–10–94	32.05	76.66	15.0	2.5
12–12–94	20–12–94	32.18	76.35	15.0	2.5
30–12–94	05–01–95	32.33	77.06	36.0	2.9
13–01–95	19–01–95	32.95	76.05	15.0	3.0
21–03–95	24–03–95	32.60	75.91	18.0	4.5
27–04–95	04–05–95	31.43	76.95	15.0	2.3
05–05–95	10–05–95	32.83	76.02	12.0	2.0
01–06–95	07–06–95	31.42	76.58	15.0	3.3
19–06–95	22–06–95	32.55	76.38	01.0	2.3
25–06–95	28–06–95	33.00	76.42	15.0	1.8
15–07–95	21–07–95	31.47	76.81	65.0	1.7
23–07–95	05–08–95	32.92	75.60	19.0	1.9

one year, a mean value of radon concentration and corresponding standard deviation were calculated. If one or more measured radon values deviated from the yearly average by more than twice the standard deviation, an event was assumed to have occurred in the respective time series. The event was called a radon anomaly if it preceded an earthquake and satisfied the above criterion. It is a purely arbitrary and relative definition. The average radon concentration level from March 1993 to August 1995 is calculated to be 5728 counts per day with a standard deviation of 4802. Results are given in Tables 1 and 2.

The first radon anomaly was recorded on 17 March 1993 under normal weather conditions. This anomaly was followed by an earthquake of a magnitude of 3.5 on 22 March 1993 in the Kangra valley. With a sudden decrease in the radon value again on 23 March, an event of 2.8 M was recorded on the same day. A radon anomaly occurred on 21 June when air and soil temperature were showing a rising trend. This anomaly was followed by a seismic event of magnitude 2.4 on 24 June in N-W Himalaya. Due to the battery failure, there are some data gaps but a number of radon spikes are evident in the radon spectrum recorded over the time window of 29 months (Figure 2). Most of these spikes are correlated with seismic events which occurred in the region as summarized in Table 3. For correlation purposes, the radon data is compared with the seismic data supplied by the Seismology Division of IMD, New Delhi which is running its network in the Kangra valley.

The Kangra valley experienced the heaviest rainfall in July and August 1993, the peak monsoon season. Radon emanation was lowest during the rainy season after heavy rainfall in the last week of June. There is a positive anomaly corresponding to the seismic event of magnitude 2.5 which occurred on 28 August. There is no seismic event during the time window September 1993 to March 1994 in the grid (32–33°N, 76–77°E) despite the fact that four radon anomalies are recorded in the data.

Radon values show large scale fluctuations during April and May 1994. The sharp peak on 2 April is followed by the seismic event of magnitude 2.5 on 10 April. However, the sharp radon peak of May 16 is not followed by any corresponding seismic event. The radon level shows a rising trend during July to October 1994 and a number of events corresponding to positive anomalies are recorded during this period. The peaks on 5 July, 19 August, 31 August, 20th September, 28th September and 9 October 1994 are followed by events of magnitude 2.9, 2.9, 3.2, 2.4, 2.2 and 2.5 on 7 July, 19 August, 8 September, 25 September, 2 and 15 October, respectively. No seismic event is recorded during November 1994 although there is a peak.

During December 1994, radon emanation was below average and does not show any positive radon anomaly. A negative peak on December 12 corresponds to an event of 2.4 M on 20 December. After this low radon value there is a sudden rise in radon emanation, impulsive increase touching its peak value on 5 January 1995. This sharp rise in activity is reflected by seismic events of magnitude 2.9 and 3.0 on 5 January and 19 January 1995, respectively. Radon fluctuations were quite predominant during February and March 1995. There was a sharp rise in the radon value on 21 March followed by an event of 4.5 M on 24 March. During May–June

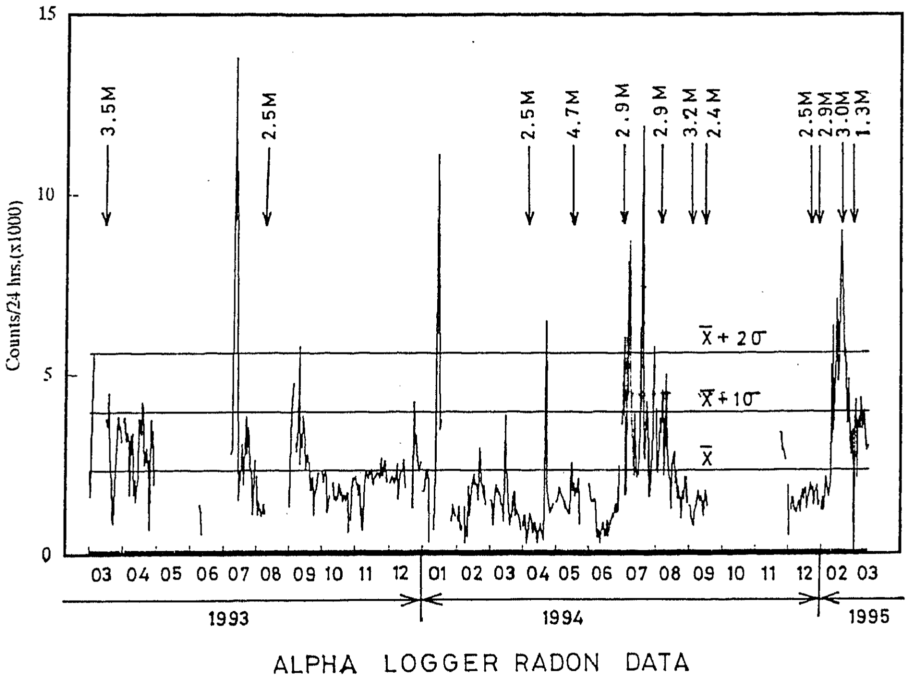

Figure 3. Alpha-logger radon data for Pathankot station (arrows represent the seismic events recorded by IMD network).

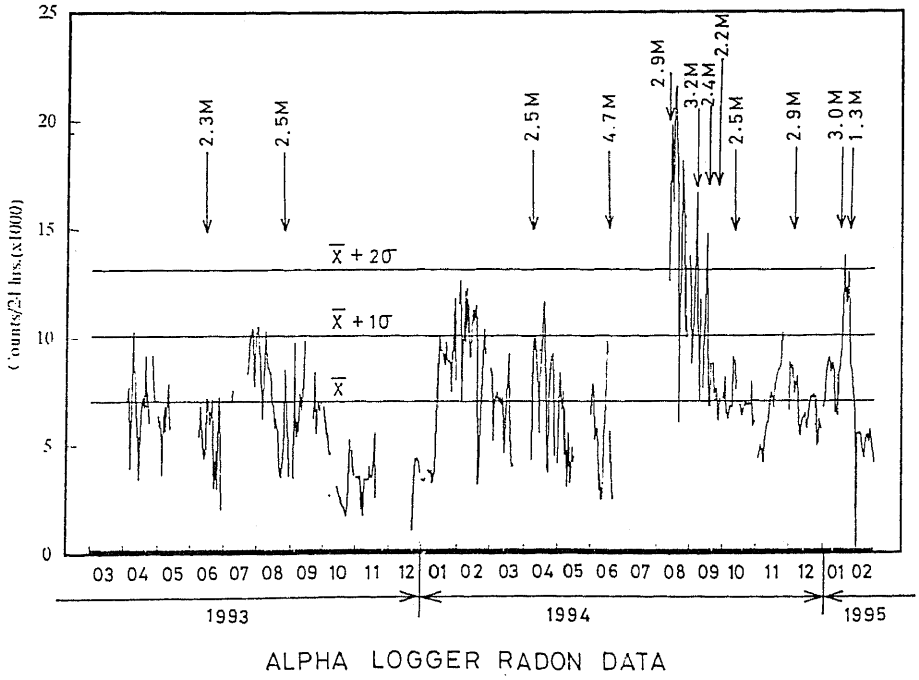

Figure 4. Alpha-logger radon data for Kotla station (arrows represent the seismic events recorded by IMD network).

radon activity shows a decreasing trend just touching average value but in July, even after the onset of the rainy season the radon value rose beyond $\bar{x}+2\sigma$. A number of radon spikes appear in the data set followed by events corresponding to these spikes (Figure 2). During August the radon value shows a decreasing trend while displaying a sharp peak left unidentified due to lack of IMD data for this period.

Alpha-logger radon data for other stations, viz. Pathankot, Kotla, Dalhausie, Chamba and Jawalamukhi are recorded as shown in Figures 3–7 respectively. Alpha-logger probe at Dalhausie was shifted to a new

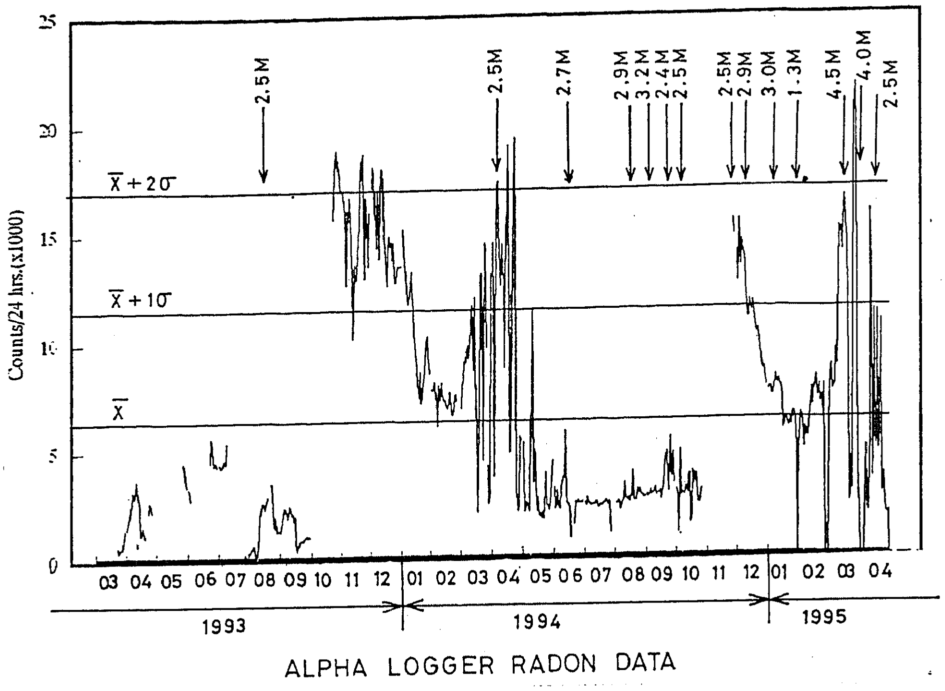

Figure 5. Alpha-logger radon data for Dalhausie station (arrows represent the seismic events recorded by IMD network).

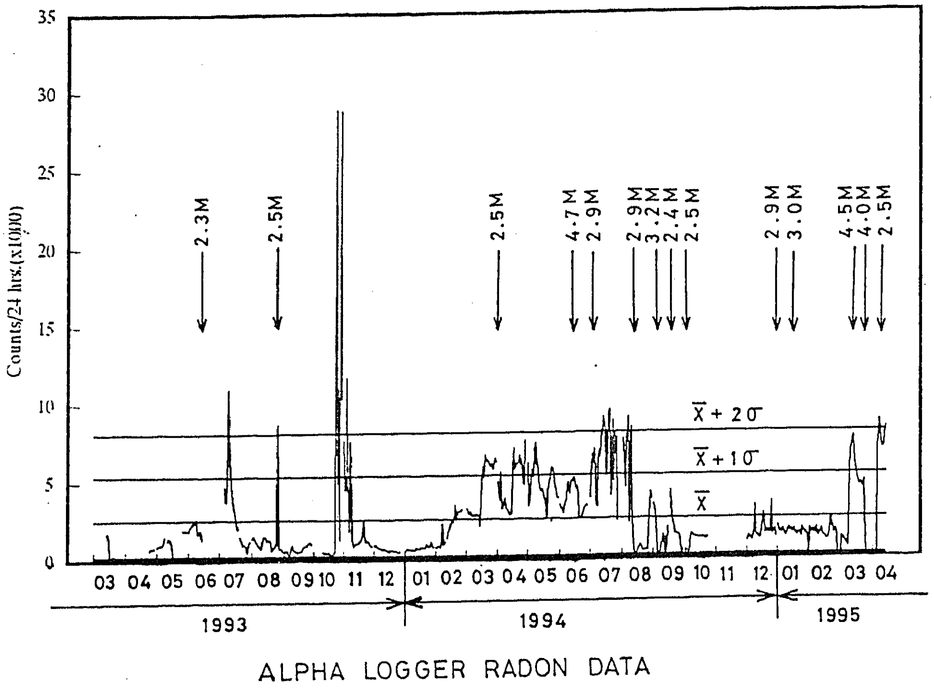

Figure 6. Alpha-logger radon data for Chamba station (arrows represent the seismic events recorded by IMD network).

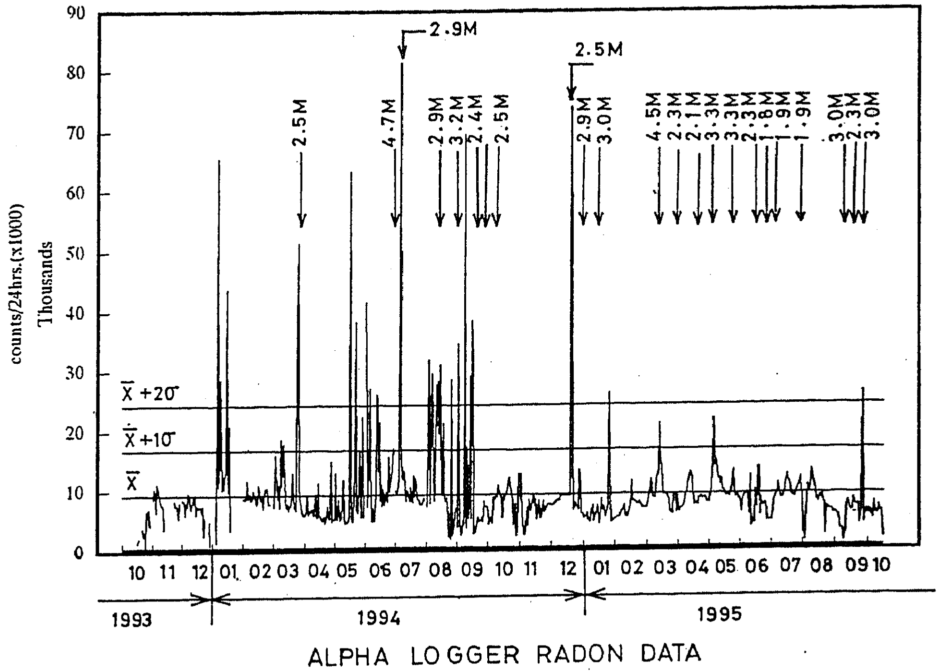

Figure 7. Alpha-logger radon data for Jawalamukhi station (arrows represent the seismic events recorded by IMD network).

site in November 1993 due to logistic problem after which it started recording radon data faithfully. Radon peaks at Dalhausie and Palampur show some matching with emanometry data reported elsewhere[11]. Jawalamukhi station is quite sensitive to seismic events occurring in the Kangra valley as shown by large-scale fluctuations in radon emission (Figure 7). Being in close proximity to a fault, it has recorded the highest average value of radon emanation. The Chamba earthquake which occurred on 24 March recorded its signature as a radon anomaly on 21 March. It is one of the unique events[13] that strongly supports radon as an earthquake precursor. The events which are correlated with alpha-logger radon data from these stations are summarized in Tables 4–8. Since there are some peaks which do not correspond to any event, a meteorological analysis of alpha-logger radon data has been carried out at Palampur station. Results of this statistical analysis are given in Table 9.

The origin and mechanism of observed radon anomalies and their relationship to earthquakes is yet poorly understood, although several constraints from laboratory experiments, mathematical modelling and *in situ* hydraulic experiments have been described[14-16]. Anderson and Grew[17] proposed the mechanism based on the stress corrosion theory. This attributes the radon anomalies to slow crack growth controlled by stress corrosion in a rock matrix saturated by groundwater. The mechanism of stress corrosion suggests that the occurrence of radon anomalies may depend on strain rate and local conditions such as rock type, elastic moduli, the pattern of microearthquakes, the degree of saturation, temperature, stress intensity factors and hydraulic properties.

Table 4. Events correlated with radon anomalies occurring at Kotla

Date of Rn anomaly	Date of event	Lat°N	Long°E	Depth (km)	M
16–06–93	24–06–93	32.86	76.02	06.1	2.3
27–08–93	28–08–93	32.54	76.76	15.0	2.5
10–04–94	10–04–94	31.55	76.76	15.0	2.5
24–06–94	02–07–94	32.72	76.05	15.0	4.7
15–08–94	19–08–94	31.72	76.63	19.0	2.9
08–09–94	08–09–94	32.54	76.43	15.0	3.2
18–09–94	25–09–94	32.22	76.47	15.0	2.4
28–09–94	02–10–94	32.64	75.86	57.0	2.2
13–10–94	15–10–94	32.05	76.66	15.0	2.5
09–12–94	20–12–94	32.18	76.35	15.0	2.5
24–12–94	05–01–95	32.33	77.06	36.0	2.9
19–01–95	19–01–95	32.95	76.05	15.0	3.0
25–01–95	04–02–95	32.50	76.00	04.0	1.3

King[18] proposed a compression mechanism for radon release. According to this mechanism the anomalous high-radon release may be due to an increase in crustal compression before an impending earthquake that squeezes out the soil gas into the atmosphere at an increasing rate. An increased outgassing rate may perturb the vertical subsurface radon concentration profile such that the deeper soil gas containing more radon is brought up to the detection level.

The Kangra valley and its environs did not experience a major seismic event in the time window, March 1993 to August 1995, except for Chamba earthquake of 24 March 1995 of magnitude 4.5 as reported by IMD network in the grid (31–33°N, 75–77°E).

Radon peaks are correlated with seismic events recorded in N-W Himalaya for all the stations using alpha-logger probes. Kotla (Table 4) shows a similar trend to Palampur station, lying in the immediate vicinity of main boundary thrust (MBT). Surprisingly, all alpha-logger sites are unique in character in response to radon fluctuations but most of the seismic events which occurred in the grid (31–33°N, 75–77°E) are reflected in the radon data. The background radon values are generally found high at Palampur, Kotla and Jawalamukhi stations which indicate the presence of radioactive minerals in this area.

Migration of radon from the source which lies between 300 and 800 m beneath the surface is only possible by groundwater flow and pressure gradient. This strain field model[16] explains the behaviour of radon migration to some extent. The variation in the sensitivity of stations towards radon emanation is due to variation in the porosity and permeability of the underlying rock strata. Seasonal variations affect the radon emanation up to 10–20% of the average value. A rise in radon value does not always mean an event. Generally in 60 to 70% of the cases, a rise between $\bar{x}+1\sigma$ and $\bar{x}+2\sigma$ corresponds to an event. The mean values of radon emanation differ widely over monitoring sites, with Chamba recording the lowest value of 2536 counts/day and Jawalamukhi, the highest radon value of 9300 counts/day. King[3] reported similar results: the average level at four sites separated only by tens of meters, differed by as much as a factor of 5. The rising trend of radon at Palampur and Jawalamukhi indicates the increase in microseismic activity in the Kangra valley (Tables 3 and 7). The low background value of radon at Chamba may be attributed to the presence of granitic rocks in the area. The other cause of its low value may be collapse of pore spaces during the process of strain field development.

Critical analysis of alpha-logger radon data (Tables 1 and 2) strongly supports the fact that radon rise does not always lead to an event, but in most of the cases

Table 5. Events correlated with radon anomalies occurring at Chamba

Date of Rn anomaly	Date of event	Lat°N	Long°E	Depth (km)	M
15–06–93	24–06–93	32.86	76.02	06.1	2.3
26–08–93	28–08–93	32.54	76.76	15.0	2.5
31–03–94	10–04–94	31.55	76.90	15.0	2.5
22–06–94	02–07–94	32.72	76.05	15.0	4.7
05–07–94	07–07–94	31.42	76.90	15.0	2.9
14–08–94	19–08–94	31.72	76.63	19.0	2.9
07–09–94	08–09–94	32.54	76.43	15.0	3.2
17–09–94	25–09–94	32.23	76.47	15.0	2.5
08–10–94	15–10–94	32.05	76.66	15.0	2.5
01–01–95	05–01–95	32.33	77.06	36.0	2.9
12–01–95	19–01–95	32.95	76.05	15.0	3.0
13–03–95	24–03–95	32.60	75.91	18.0	4.5
25–03–95	30–03–95	33.23	75.96	24.0	4.0
06–04–95	06–04–95	33.23	75.07	15.0	2.5

Table 6. Events correlated with radon anomalies occurring at Pathankot

Date of Rn anomaly	Date of event	Lat°N	Long°E	Depth (km)	M
19–03–93	22–03–93	32.04	76.06	15.0	3.5
09–08–93	28–08–93	32.54	76.76	15.0	2.5
05–04–94	10–04–94	31.55	76.90	15.0	2.5
20–05–94	02–07–94	32.72	76.05	15.0	4.7
03–07–94	07–07–94	31.42	76.90	15.0	2.9
11–08–94	19–08–94	31.72	76.63	19.0	2.9
05–09–94	08–09–94	32.54	76.43	15.0	3.2
15–09–94	25–09–94	32.23	76.47	15.0	2.4
17–12–94	20–12–94	32.18	76.35	15.0	2.5
26–12–94	05–01–95	32.33	77.06	36.0	2.9
19–01–95	19–01–95	32.95	76.05	15.0	3.0
29–01–95	04–02–95	32.50	76.00	04.0	1.3

Table 7. Events correlated with radon anomalies occurring at Jawalamukhi

Date of Rn anomaly	Date of event	Lat°N	Long°E	Depth (km)	M
25–03–94	10–04–94	31.55	76.90	15.0	2.5
30–06–94	02–07–94	32.72	76.05	15.0	4.7
05–07–94	07–07–94	31.42	76.90	15.0	2.9
15–08–94	19–08–94	31.72	76.63	19.0	2.9
02–09–94	08–09–94	32.54	76.43	15.0	3.2
17–09–94	25–09–94	32.23	76.47	15.0	2.4
25–09–94	02–10–94	32.64	75.86	57.0	2.2
11–10–94	15–10–94	32.05	76.66	15.0	2.5
20–12–94	20–12–94	32.18	76.35	15.0	2.5
29–12–94	05–01–95	32.33	77.06	36.0	2.9
15–01–95	19–01–95	32.95	76.05	15.0	3.0
15–03–95	24–03–95	30.60	75.91	18.0	4.5
01–04–95	04–04–95	31.43	76.95	15.0	2.3
24–04–95	01–05–95	32.33	76.61	53.0	2.1
06–05–95	12–05–95	32.40	76.43	02.0	3.3
26–05–95	07–06–95	31.42	76.58	15.0	3.3
18–06–95	22–06–95	32.91	76.38	01.0	2.3
26–06–95	28–06–95	33.00	76.42	15.0	1.8
07–07–95	08–07–95	33.61	76.54	15.0	1.9
01–08–95	05–08–95	32.92	75.60	19.0	1.9
10–09–95	15–09–95	32.62	76.73	03.0	3.0
17–09–95	20–09–95	32.81	76.28	05.0	2.3
28–09–95	29–09–95	32.61	76.56	06.0	3.0

Table 8. Events correlated with radon anomalies occurring at Dalhausie

Date of Rn anomaly	Date of event	Lat°N	Long°E	Depth (km)	M
26–08–93	28–08–93	32.54	76.76	15.0	2.5
06–04–94	10–04–94	31.55	76.90	15.0	2.5
21–06–94	02–07–94	32.72	76.05	15.0	4.7
18–08–94	19–08–94	31.72	76.63	19.0	2.9
05–09–94	08–09–94	32.54	76.43	15.0	3.2
22–09–94	25–09–94	32.23	76.47	15.0	2.5
05–10–94	15–10–94	32.05	76.66	15.0	2.5
06–12–94	20–12–94	32.18	76.35	15.0	2.5
24–12–95	05–01–95	32.33	77.06	36.0	2.9
09–01–95	19–01–95	32.95	76.05	15.0	3.0
02–02–95	04–02–95	32.50	76.00	04.0	1.3
17–03–95	24–03–95	32.60	75.91	18.0	4.5
28–03–95	30–03–95	33.23	75.96	24.0	4.0
06–04–95	06–04–95	33.23	75.07	15.0	2.5

Table 9. Statistics of radon-monitoring site and its correlation with meteorological parameters

Parameter	Avg.	Std.dev.	Avg + std	Percentage vari. coefficient (C%) Std./Avg.	Correlation coefficient
Radon (Counts/24 h)	5728	4802	15330	83	–
Evaporation (mm)	2.65	2.53	7.71	95	– 0.08
Temperature (°C)	22.01	12.17	46.35	55	0.10
Relative humidity (%)	47.56	20.19	87.94	42	0.12
Wind velocity (km/h)	5.53	1.76	9.05	32	– 0.14
Rainfall (mm)	40.57	56.38	153.33	138	0.08

it precedes the occurrence of earthquakes. Although the concept of negative peak is there, there is no exact demarcation, from where and to what level the value will decrease. A low or high value in radon emanation may be due to meteorological variations too. The results reveal that radon emanation is directly correlated with temperature, relative humidity and rainfall, and inversely with evaporation and wind velocity (Table 9).

Finally, we may conclude that there is a high microseismic activity in the region and the stations in close proximity of MBT record impulsive values of radon before an impending seismic event. Sensitivity of a radon-monitoring station also depends upon the geological conditions of the region, nature of the soil and the meteorological variations.

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A feasibility study towards absolute dating of Indo-Gangetic alluvium using thermoluminescence and infrared-stimulated luminescence techniques

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Results of a successful maiden attempt to date the Indo-Gangetic alluvium using the luminescence dating technique are presented. The low equivalent dose for the surface sample indicates that these samples had experienced a solar resetting of geologically acquired luminescence. The infrared stimulated luminescence ages on other terraces range from ~ 2 to 15 ka and are stratigraphically consistent.

THE 0.25 million sq km Gangetic plain is a foreland basin, extending from Delhi ridge in the west to Rajmahal hills in the east and from Siwaliks in the north to Bundelkhand–Vindhyan high-lands in the south. Major rivers on this plain, viz. Ganga, Yamuna, Ramganga, Ghaghra, Gandak, etc. originate in the Himalaya and carry a large sediment load towards Bay of Bengal. The alluvial fill shows a south-eastward decrease in thickness[1] and this varies from 1000 m in the north near Himalaya to < 10 m in the south adjoining peninsular shield. The basement of the Ganga basin is segmented by a number of transversely occurring geofractures, giving a horst and graben topography[2]. This structural fabric of the basin was responsible for the creation of a number of sub-basins such as the Western UP Shelf, Faizabad

Ridge, Eastern UP Shelf, etc. Evidences indicate that during Quaternary, reactivation of these geofractures resulted in the neotectonic adjustments, leading to a relative sinking and uplifting of the different blocks as manifested by morphologic changes of rivers such as meander cut-off, straightening of course and migration of confluence. This scenario suggests that the alluviation dynamics was controlled by them. Table 1 provides a summary of the lithostratigraphy of the Ganga plain in UP[3-5].

The Gangetic plain of UP is a thick pile of Quaternary sediments overlying a northerly slopping basement[2,6], comprising rocks of Bundelkhand gniessic complex (BGC), Delhi Group, Vindhyan Group and Siwalik Group. The Quaternary alluvium for which the dating has been attempted, has been classified into Older Alluvium comprising Banda and Varanasi Alluvial deposits. The New Alluvium consists of fan deposits near the foothills of Himalaya and, terrace and recent alluvial deposits within the valley fills of present day rivers.

The flood plain restricted to the present day valley of the rivers has been classified into older flood plain, comprising erosional terrace, depositional terrace (T_1) and active flood plain (T_0) representing present day meander belt of the rivers[7]. These aggradational surfaces are of regional character and possess a Holocene top soil[8]. These structures have been suggested to be formed as a result of variation in the gradient of river streams in response to eustatic sea level changes[9]. Relying on this model, Singh[8,9] proposed the formation age of the oldest geomorphic unit, the Varanasi Plain to be ~ 128 ka, representing the last interglacial event, and the age 30–25 ka BP for the formation of the terrace T_1 during the succeeding high sea level stand. The relative dependence of sedimentation rate of the alluvium on neotectonic and climatic events (sea level changes) has still remained unresolved owing to the absence of any reliable dating framework. The only attempt so far to directly date these terraces has been the radiocarbon dating of carbonate nodules embedded in the terrace sediments, which range from 9 to 40 ka (ref. 10). However, radiocarbon ages of the carbonates are often suspect due to contamination either from the modern carbon or the dead carbon[11]. Therefore, in the present paper we examine the feasibility of dating the terrace deposits by the recently developed dating techniques, viz. the thermoluminescence (TL) and infrared stimulated luminescence (IRSL) dating methods. The basic advantage of these methods is that they utilize the minerals constituting the sediment for estimation of the depositional event.

Luminescence dating is radiation microdosimetry of natural radiation environment. All sediments contain radionuclides, viz. ^{238}U (in ppm), ^{232}Th (in ppm) and ^{40}K (in %). The radiations arising from the decay of these radionuclides along with the cosmic rays provide a constant source of natural radiation flux. The interaction of these radiations with crystal lattice of mineral generates an avalanche of electron and holes. During their motion in crystal lattice, a small fraction of these charges gets trapped at lattice defect sites. The binding energy of the trapped charges at some of these sites is sufficiently high to permit a residence time of trapped charges extending to 10^6–10^8 a at room temperature. This implies that the total number of trapped charges increase with time until a thermal/optical stimulus provides sufficient energy for their detrapping. A small fraction of the detrapped charges radiatively recombine to produce luminescence which bears a proportional relationship to the total radiation exposure. The evaluation of sediment age (t) by TL/IRSL involves measurement of the environmental dose rate (R) and the total dose deposited in mineral (Q). The total dose that accumulated in the mineral grain in the form of total trapped charge concentration is deciphered by measuring the natural luminescence and calibrating it with the luminescence

Table 1. Summary of Quaternary lithostratigraphy of the Ganga plain

Age	Lithostratigraphic units including thickness in metres	Lithology and distribution
	New alluvium	
Holocene	Recent alluvium 5–10 m	Confined to present bank limits of the present day rivers comprising bar sand and occasional silt cover.
	Terrace alluvium 10–30 m	Occurring in the terraced valley zones of the present rivers. Comprises alternate sequence of silt and sand.
	Fan alluvium 5–15 m	Coarser clastics with pebbles of all sizes in a radiating channel pattern near the Siwalik Foot Hill.
	Older alluvium	
Lower to upper Pleistocene	Varanasi alluvium > 600 m	Polycyclic sequence of oxidized silt-clay and micaceous sand with ferruginous nodules and kankar dissiminations. Covering most part to the north of Ganga–Yamuna axis from Ghaziabad to Ballia. Derived from Himalaya.
	Banda alluvium 120–300 m	Comprises a sequence of silt clay and red quartzo-feldspathic sand with occasional kankar. Covering parts of Bundelkhand and Baghelkhand regions to the south of Ganga–Yamuna developed by peninsular shield.

sensitivity of the sample (i.e. luminescence/unit dose). The age is then calculated by dividing the estimated dose (Q) with the environmental dose rate (R).

$$\text{Age } (t) = \frac{\text{Total luminescence}}{(\text{Luminescence/unit dose}) \times (\text{Dose/year})}$$

$$= \frac{Q}{R}. \qquad (1)$$

In the case of sediments, the event dated by luminescence method is the most recent episode of sunbleaching. It is considered that during the predepositional weathering and transport, the minerals are exposed to the sunlight and this exposure causes a photobleaching of 'geological luminescence' to a near 'zero' residual value I_0. The geological luminescence reflects the signal acquired by the mineral during the geological antiquity. On sedimentation and consequent burial, the sun exposure ceases and a fresh accumulation of luminescence (over and above the residual level) I_0 is initiated because of the irradiation from ambient radioactivity. The total luminescence level I_{nat} is related to the luminescence level (I_d) acquired since sedimentation through the relation

$$I_{\text{nat}} = I_0 + I_d \qquad (2)$$

and the luminescence age of depositional event is given by

$$\text{Age} = \frac{D(I_d)}{R}, \qquad (3)$$

where $D(I_d)$ is the equivalent laboratory radiation dose required to generate the signal I_d in the bleached sample. An important aspect of application of the luminescence dating is to ascertain the extent of photobleaching by predepositional sunbleaching. For sediment transported in air through suspension or by saltation, the duration of transport and the availability of un-attenuated daylight flux ensures that maximum possible bleaching of the luminescence to a residual value I_0 occurs. On the other hand, sediments transported fluvially, receive an attenuated solar flux due to turbulence in the water and the sediment load[12-14] (see Figure 1). Thus, additional experiments are needed to ascertain the level of photobleaching. These include laboratory bleaching studies using filtered sunlight[15,16] and study of sediments recently deposited under a depositional environment identical to that of the sample[12]. A more recent approach has been to conduct single grain analysis to identify the most bleached mineral grains in a complex suite of samples with grains of different daylight exposure history[17].

Because Indo-Gangetic alluvium sediments are also fluvially transported, two types of experiments, viz. the infrared stimulated luminescence (IRSL) dating and the partial bleach thermoluminescence (TL) analysis were performed. In the IRSL technique, a stimulation with 880 nm source was used. This excitation probes the most sensitive optically stimulable signal[18]. Figure 2 shows the bleaching rate of IRSL of a 90–150 μm K-feldspar mineral separated from the Gangetic alluvium sample on sunlight exposure. It is seen that sunlight exposure can erase 90% of the infrared stimulable signal in few tens of seconds.

Samples for the present study were collected by B. K. Bisaria from different geomorphic surfaces from Budaun,

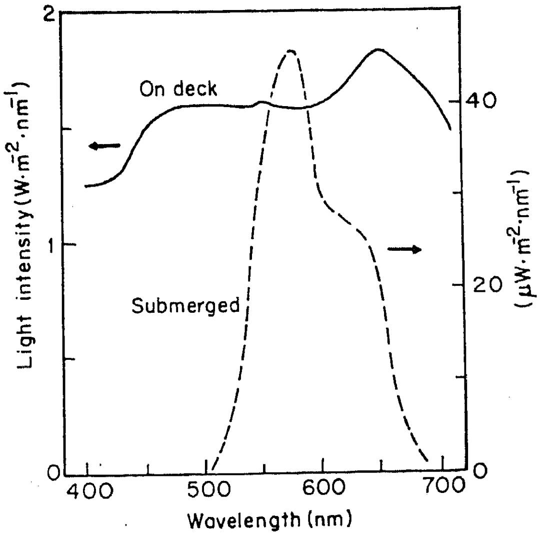

Figure 1. Comparison of sunlight spectrum on deck and at 4 m depth in a turbid river. The intensity at 4 m depth is ~ 10^4 times less than that at the surface. Note also the spectral attenuation below 500 nm and above 690 nm (from Berger and Luternauer[15]).

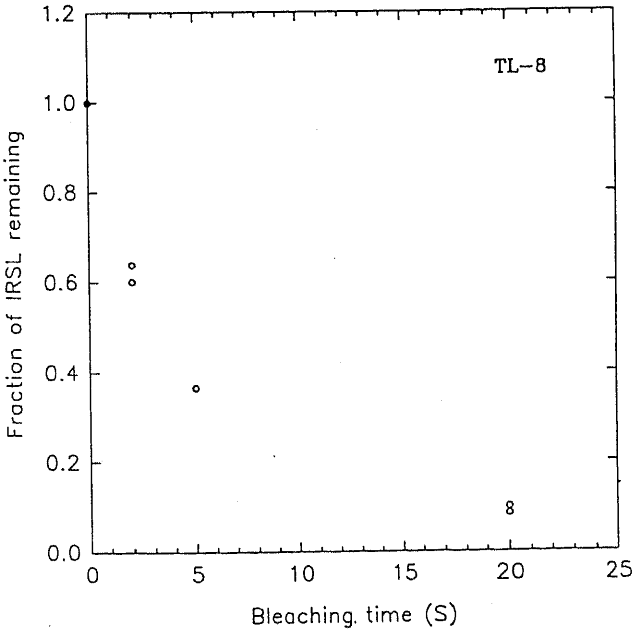

Figure 2. Bleaching of IRSL with sun exposure, expressed as fraction of IRSL remaining for the sample TL-8.

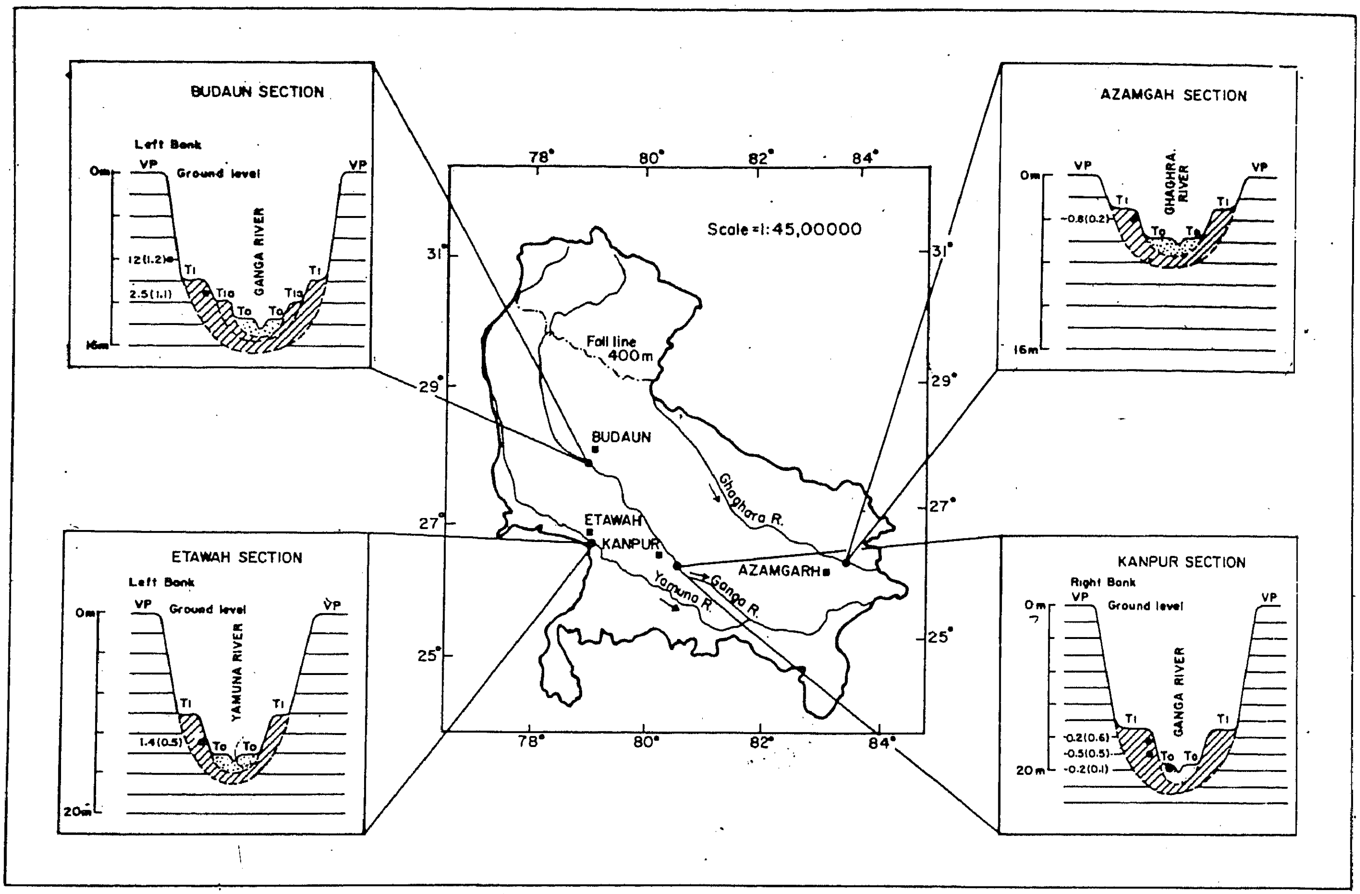

Figure 3. Location of sampling sites, stratigraphic position of sample and IRSL ages.

Table 2. Sample, stratigraphic position, dosimetry data, equivalent dose and luminescence age estimates of samples from Indo-Gangetic alluvium

Sample				Radioactivity data			Dose rate (Gy/ka)			Equivalent dose		Age (ka)	
Code	Site	Terrace	Depth (mbas)	Th (μg/g)	U (μg/g)	K (%)	Internal*	External†	Total dose	TL (Gy)	IRSL (Gy)	TL	IRSL
TL-8	Budaun (North)	T_2	8.0	5.5(1.1)	1.6(0.3)	2.37	0.19	2.86	3.0(0.3)	58(11)	36.5(0.8)	19.0(4.0)	12.0(1.2)
TL-6	Budaun (North)	T_1	11.4	4.7(1.5)	2.1(0.4)	1.08	0.09	2.21	2.3(0.2)	12(16)	5.8(3.0)	5.2(5.8)	2.5(1.1)
TL-34	Etawah (North)	T_1	12.9	14.5(4.6)	3.1(1.1)	1.67	0.13	4.00	4.1(0.3)	14(3)	5.7(2.5)	3.4(0.7)	1.4(0.5)
TL-5	Azamgarh	T_1	4.0	7.1(1.6)	1.8(0.5)	2.40	0.19	3.44	3.6(0.3)	1.5(3)	3.0(0.8)	0.4(0.7)	0.8(0.2)
5/92	Kanpur (R.Bank)	T_1	16.4	13.8(3.9)	2.8(1.2)	1.24	0.10	3.49	3.6(0.2)		0.58(2.9)		0.2(0.6)
6/92	Kanpur (R.Bank)	T_1	18.0	6.1(1.4)	1.9(0.4)	1.11	0.09	2.30	2.4(0.2)		1.3(1.5)		0.5(0.5)
4/92	Kanpur (R. Bank)	T_0	19.7	7.1(2.5)	2.1(0.7)	1.3	0.10	2.63	2.7(0.2)		0.55(0.4)		0.2(0.1)

* For unetched grains external alpha dose estimated by assuming a value as 0.2 ± 0.1.
Cosmic ray dose assumed as 150 μGy/a.
Average 10% moisture content assumed for correcting dose attenuation.
† Internal dose rate estimated by assuming K content as 10%.
The numbers in the parenthesis provide error in the measurement. Thus, 12.0(1.2) is 12.0 ± 1.2.

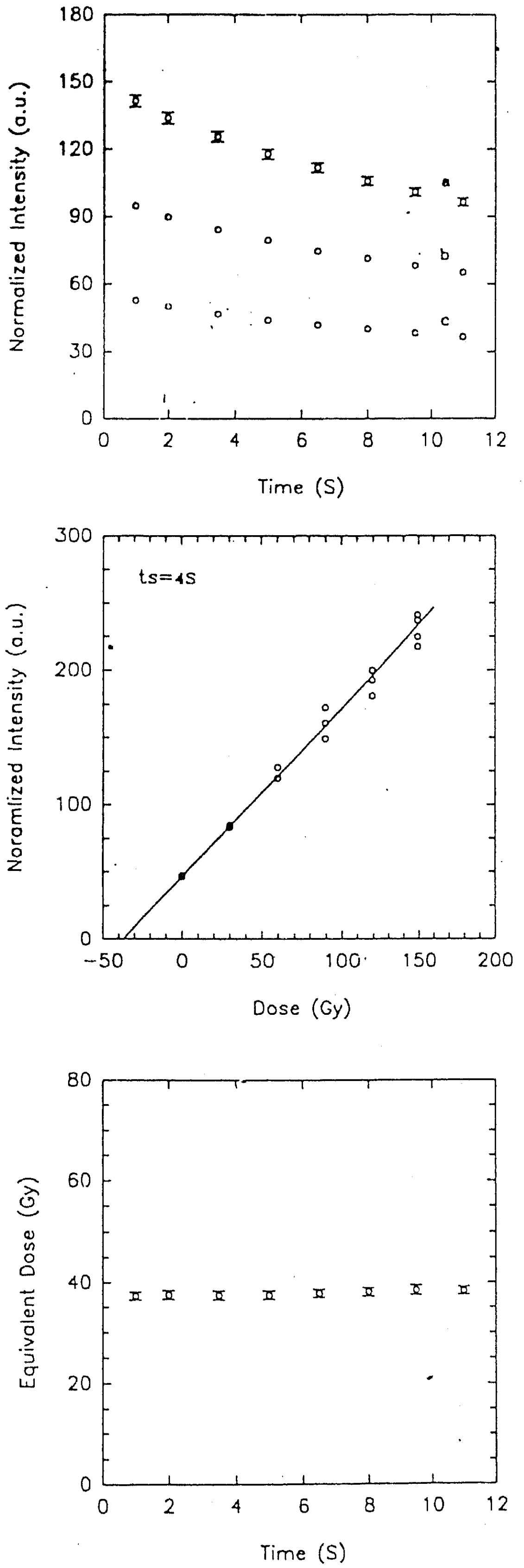

Figure 4. Typical IRSL shine curves, growth curves and equivalent dose plateau for K-feldspar mineral separates for sample TL8. The IRSL shine curves are for (a) natural + β-dose (60 Gy), (b) natural + β-dose (30 Gy) and (c) natural aliquot.

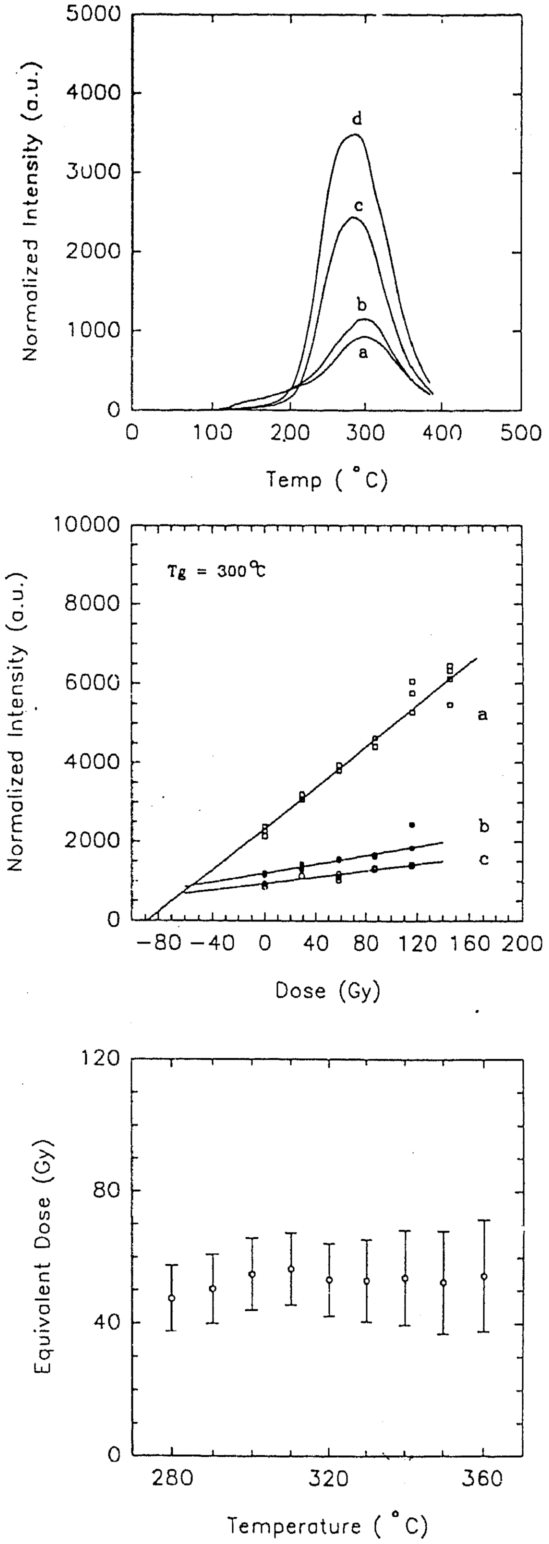

Figure 5. Plot of typical glow curve (top box), growth curve (middle box) and the equivalent dose plateau (bottom box) for K-feldspar mineral separate for sample TL8. The glow curves are for (a) natural + sun bleach (10 min), (b) natural + sun bleach (5 min) and (c) natural aliquot and (d) natural + 30 Gy beta dose. The partial bleach growth curves are for (a) natural + sun bleach (5 min) and (b) natural + sun bleach (10 min). The equivalent dose plateau is for 5 min sun bleach.

Kanpur and Etawa sites in the central region of the Indo-Gangetic plain. Figure 3 shows the sampling localities and the cross-sections of fluvial deposits. The samples were collected as horizontal cores in metal cylinders from freshly exposed vertical cuts. The samples were pre-treated with 1N HCl and 30% H_2O_2 to remove carbonate and organics. The sediment grains of size 90–150 μm were then separated by dry sieving. Feldspar was isolated by density separation (< 2.58 g/cm^3) using a sodium polytungstate compound. Due to the limited sample availability, HF etching of the alpha-exposed layer of these samples, except for BKB-8, could not be attempted. Several monolayer aliquots were obtained from the sample by sprinkling the extracted feldspars onto stainless steel discs, containing silkospray for adhesion.

Irradiations were performed using beta source ^{90}Sr/^{90}Y of strength 30 mCi with dose rate of ~ 0.05 Gy/s. Luminescence from grains was observed using Corning 5-58+7-59 blue transmitting filters combined to an EMI 9635 QA PMT photomultiplier and a photon counting system. For infrared stimulation, an indigenous semi-automated system employing a source made by an array of 16 TEMT 484 diodes, emitting at 880 ± 80 nm was used. The luminescence output from the individual discs was normalized using integrated luminescence obtained from the natural samples on short infrared stimulation for 1 s. Prior to the measurements, the samples were pre-heated for 160°C for 330 min to remove the unstable luminescence signal that is additionally generated during the laboratory irradiation and bleaching procedures. In the analysis, the samples were measured for IRSL for 15 s and then for their TL. This was done on the basis of IRSL stimulation causing an insignificant (~ < 2%) depletion of TL signal[19]. In the analysis, the luminescence intensity vs radiation dose growth curves were fitted to linear regressions. In estimating the dose rate, uranium and thorium concentrations were measured using thick source alpha counting and potassium was estimated by atomic absorption spectroscopy. The concentrations were converted into dose rates using conversion table provided in Aitken[20] (assuming the decay series to be in radioactive equilibrium). An average 10% water content and 150 μGy/a cosmic ray dose was assumed in calculating the dose rates. The internal β-dose has been calculated using the factor provided by Mejdahl[21] and assuming a 10% internal potassium content. For the unetched samples, the alpha-dose has been estimated assuming an alpha efficiency of 0.2 ± 0.1 (ref. 22). The error in age was estimated using the prescription of Aitken[20].

Table 2 provides a summary of TL and IRSL data and Figures 4 and 5 provide typical shine down curve, growth curve and plateaux for IRSL and glow curve, growth curve and plateaux for TL data. Both the TL and IRSL ages show a stratigraphic consistency. These ages are also concordant with the previous TL age estimates on similar terrace sequences from Roorkee area[12]. Note that the age 200 years obtained on surface sediment suggests that the luminescence signal is definitely bleached prior to burial and this age also can be taken as the lower limit to the ages obtainable by the IRSL technique. The age 200 years for surface samples can also be considered as the over-estimation amount for all the samples deposited under similar conditions. From Table 2 it is apparent that TL ages are somewhat higher compared to their IRSL ages. In view of easy bleachability of IRSL and the near zero IRSL age of the surface sample, we consider IRSL ages to be more realistic compared to the corresponding TL ages. The age of the Varanasi Plain at Budaun site is obtained as 12 ka is consistent with the radiocarbon age of ~ 12 ka obtained at this depth deduced from the data from Kanpur and Fatehpur sequences (Figure 2 of Rajgopalan[10]). The ages on terrace T_1 suggest their formation during late Holocene period. The low TL/IRSL ages on T_1 and Varanasi Plain suggest either that these terraces are substantially younger than the inferred age[8,9], or that they are part of the Holocene aggradation deposits. The ages arrived at for Varanasi Plain and T_1 terraces are in concordance with evolutionary model of the Ganga Plain as suggested by Bisaria *et al.*[7]. More detailed chronological work is needed to conclude the age bracket for these terraces.

The preliminary dating results show that the zeroing of luminescence signal indeed occurred for these samples and that the ages are stratigraphically consistent, demonstrating the reliability of the technique and its future prospects in examining the time evolution of the Indo-Gangetic sequence.

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Record of the Cretaceous magnetic quiet zone: A precursor to the understanding of evolutionary history of the Bay of Bengal

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Magnetic study along a transect joining the sites of Ocean Drilling Programme (ODP) leg 116 (1°S, 81°24′E) and the vicinity of Deep Sea Drilling Project (DSDP) site 218 (7°N, 87°30′E) revealed the presence of a wide magnetic smooth zone sandwiched between the known Late Cretaceous anomaly A34 (≈ 84 Myr) and the younger magnetic anomaly sequence of Early Cretaceous crust, represented by M0 (≈ 118 Myr). The smooth magnetic zone seems to have evolved during the Cretaceous long normal polarity epoch (superchron K–T) 118–84 Ma and is usually referred to as the Cretaceous magnetic quiet zone. Identification of this zone in the distal Bengal Fan perhaps serves as a missing link between the Early Cretaceous and the Late Cretaceous evolution of the crust and establishes a continuous evolutionary record of the Bay of Bengal since India's breakup from the eastern Gondwanaland continents.

SEVERAL plate reconstruction models have been proposed for the breakup of Pangea and the eastern Gondwanaland, in which the east coast of India and the Enderby Landmass of Antarctica were juxtaposed and the western margin of Australia lies east of Greater India during the Late Jurassic[1–11]. The breakup of India from the Antarctica–Australian plate sets the stage for the evolution of the Bay of Bengal and the eastern Indian Ocean. The Bengal Fan is one of the thickest sedimentary basins of the world[12–15]. Powell *et al.*[7] were of the view that the magnetic anomalies will be hard to find off the east coast of India due to huge sediment accumulation. Though extensive underway geophysical data was collected during the International Indian Ocean Expedition Programme (1959–1965) and subsequent expeditions to unravel the evolutionary history of the Indian Ocean[16–19], not much was known about the age and nature of the ocean floor of the Bengal Fan[6,7,20,21]. Various plate reconstruction models[5,7,22] show the stage-by-stage evolution of the eastern Indian Ocean since the Late Cretaceous (84 Myr) to the Present. However, refined plate reconstruction models for the early opening prior to Late Cretaceous are poorly constrained[10] due to inadequate geophysical data, particularly in the Bay of Bengal, northeastern Indian Ocean.

Recent geophysical studies[23,24] in the northern Bay of Bengal revealed the presence of N30°E trending Mesozoic magnetic anomaly sequence M11 to M0 (corresponding 132.5 to 118 Myr age old crust) and N120°E (≈ NW–SE) trending fracture zones (Figure 1). The direction of these fracture zones indicates the initial motion of the Indian plate from Antarctica–Australia immediately after the Early Cretaceous breakup. In the present study, a smooth magnetic field has been observed on a transect joining the sites of ODP leg 116 and the vicinity of DSDP site 218 over a distance of about 415 km continuing from the known Late Cretaceous anomaly A34 (≈ 84 Myr) and culminating with the Early Cretaceous crust. This magnetic smooth zone is significant for establishing the continuous evolutionary history of the Bay of Bengal since India's separation from the eastern Gondwanaland during the Early Cretaceous.

About 1200 line km of bathymetry, magnetic, gravity and multichannel seismic reflection data along the transect joining the sites of ODP leg 116 and the vicinity of DSDP site 218 have been collected onboard *ORV Sagarkanya* during 1995 (April–May). The processed data (Figure 2) has been used to infer the nature and age of the crust in the distal Bengal Fan to better constrain the evolutionary history of Bengal Fan.

The identification of the Early Cretaceous crust[23,24] and the Late Cretaceous crust[5,22] in the northern and southern Bay of Bengal respectively suggests that the ocean floor of the Bay of Bengal should have a continuous evolution record since the Early Cretaceous. This can be established either by the sampling of the oceanic basement rocks or by the study of seafloor spreading type of magnetic anomalies. We have used the total intensity earth's magnetic field data to infer the nature of the basement. The observed magnetic

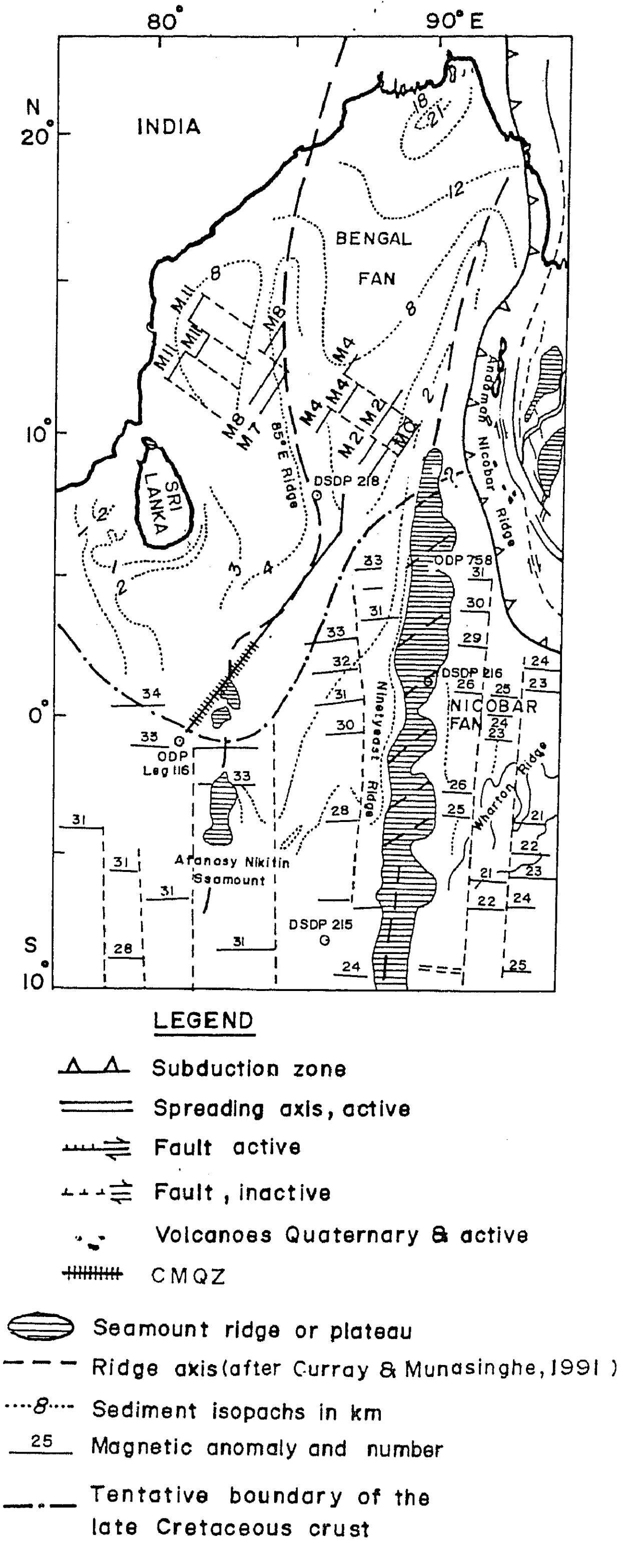

Figure 1. Tectonic summary chart of the northeastern Indian Ocean and the Bay of Bengal[20]. The Mesozoic magnetic anomaly lineations and NW–SE trending fracture zones dominate the northern Bay of Bengal[23]. The thick line joining the sites of ODP leg 116 and the vicinity of DSDP site 218 represents the multisensored geophysical transect (gravity, magnetic, bathymetry and multichannel seismic reflection data). The 415 km extent of the Cretaceous magnetic quiet zone (CMQZ) sandwiched between the Early Cretaceous and Late Cretaceous crusts has been shown with hatching.

record (Figure 2) is characterized with magnetic anomaly 34 signature at the sites of ODP leg 116, about 415 km wide smooth magnetic field with subdued amplitudes (< 50 nT) and chain of magnetic anomalies with 200–600 nT amplitude towards northeastern end of the profile. The smooth magnetic field culminates with a positive magnetic anomaly, which, perhaps, represents the younger anomaly M0 (≈ 118 Myr) of the Mesozoic sequence. The crust between the magnetic anomaly isochrons M0 and 34, evolved during the Cretaceous long normal polarity epoch (superchron K–T), corresponds to 118–84 Myr and has been commonly referred to as the Cretaceous magnetic quiet zone (CMQZ)[25]. Roots and Srivastava[26] quoted that the mechanism for occurrence of such magnetic smooth/quiet zones could be due to (i) period of non-reversal of the Earth's magnetic field[27] (ii) magnetization of the seafloor close to the magnetic equator[28] (iii) spreading being slow, the anomalies produced were too narrow to be individually resolved at the sea surface[29] and (iv) initial spreading between margins being markedly oblique to the spreading direction with the consequent fragmentation of the ridge into short spreading segments and the resultant production of magnetic source blocks too small to be resolved at the sea surface[30]. During this Cretaceous long normal polarity interval major plates reorganization has also been reported[4,7,21].

Analysis of the geophysical data revealed a distinct record (Figure 2) of about 415 km wide magnetic smooth zone prior to anomaly 34 (≈ 84 Myr) in the distal Bengal Fan. Occurrence of this magnetic smooth zone prior to the Late Cretaceous magnetic anomaly isochron 34 suggests that this magnetic smooth zone is sandwiched between the Early Cretaceous and Late Cretaceous crusts. We attribute this magnetic smooth zone to CMQZ as mentioned earlier. The crude estimates reveal that the crust of the CMQZ seems to have evolved with a half-spreading rate of 1.2 cm/yr. Similar order of widths and spreading rates prevailed over the magnetic smooth zones elsewhere in the world oceans[25]. The estimated half spreading rate of 1.2 cm/yr for this magnetic profile may be apparent since the direction of spreading during the Middle Cretaceous was not known. At the same time, the orientation of this profile seems to be nearly parallel to the Early Cretaceous spreading ridge between India and contiguous Antarctica–Australian plates. Roots[30] offered an explanation that the initial spreading between the margins that were markedly oblique to the spreading direction results in the fragmentation of the ridge into short-spreading segments. The resultant production of magnetic source blocks became too small to be resolved at the sea surface by magnetic observations. Hence, low amplitude or smooth magnetic field would result due to oblique spreading. The observed magnetic profile (Figure 2) is distinctly characterized by such a smooth zone, the known magnetic signature of anomaly

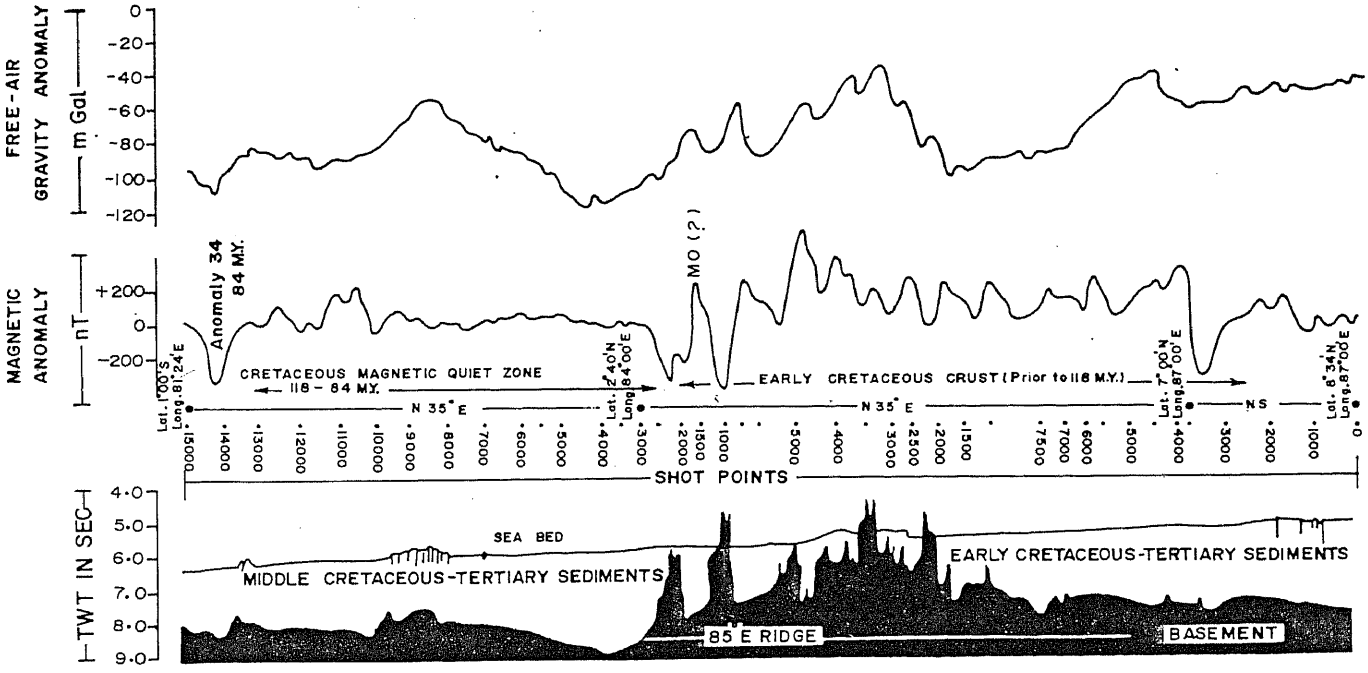

Figure 2. Composite geophysical anomaly map along the transect joining sites of ODP leg 116 and the vicinity of DSDP site 218. Part of the transect crosses the axis of the 85°E Ridge. The free-air gravity anomaly profile is characterized by long wavelength lows most probably corresponding to the 85°E Ridge[34]. The free-air gravity profile is superposed with high frequency anomalies corresponding to the irregular pinnacles of the exposed basement. The magnetic anomaly profile from southwest to northeast depicts the Late Cretaceous crust (magnetic isochron 34, ≈ 84 Ma), Cretaceous magnetic quiet zone (84–118 Myr) and the Early Cretaceous crust influenced partly by the 85°E Ridge. The line drawing of the unprocessed near trace record represents the seabed and the acoustic basement most probably the real basement. The basement of the CMQZ is relatively smooth and deeper.

34 (≈ 84 Myr) and the Early Cretaceous seafloor spreading type magnetic anomalies. The estimated average spreading rate for the Early Createaceous crust in the Bay of Bengal was 3.4 cm/yr (refs 23, 24). The two different spreading rates, i.e. 3.4 cm/yr for the Early Cretaceous crust represented by Mesozoic anomaly sequence M11–M0 (133–118 Ma) and the Cretaceous quiet zone (118–84 Ma) suggest that the seafloor spreading process slowed down during the K–T superchron with simultaneous major plates reorganization (India, Antarctica–Australia). Poehls *et al.*[25] reported similar slow-spreading rates (1.5 cm/yr) for the north Atlantic smooth zone.

The basement of the CMQZ, mapped from the seismic reflection profile in the distal Bengal Fan, occurs at about 8–9 km from the sea surface with an average sediment thickness of 3–4 km (Figure 2). This basement is relatively smooth. The CMQZ culminates with the crust of magnetic isochron 34 (≈ 84 Myr) at the sites of ODP leg 116, while towards north it merges with the Early Cretaceous crust of about 118 Myr (Figure 2). The boundaries of the CMQZ are marked by a sharp difference in the magnetic field. This may be an edge effect and is due to the amalgamation of CMQZ crust with the two different ages of the crust in the Bay of Bengal. The crust of the CMQZ between the magnetic anomaly isochrons M0 and 34 (118–84 Ma) near the northern exposure of the Ninetyeast Ridge (Figure 1) appears to be affected by the subduction processes, resulting in a narrower width of CMQZ. This part of the CMQZ crust in the Bay of Bengal may be an exception so far as the general width of the CMQZ in question is concerned.

The eastern Gondwanaland plate reconstruction models presented hitherto[5,7,9,10] seem to be incomplete due to lack of information about the age of the crust and trends of fracture zones in the Bengal Fan prior to the Late Cretaceous. This led to suggest India's breakup from the eastern Gondwanaland around magnetic isochron M0 (118 Myr) (ref. 10) and around M4 (126 Myr) (ref. 20). Further, a small segment of magnetic anomaly zone off the east coast of India has been interpreted as a magnetic smooth zone[31] prior to the Mesozoic anomalies. Banerjee *et al.*[32] have also interpreted the presence of Barremian or younger oceanic crust in the Bay of Bengal.

Mapping of considerably wide CMQZ (≈ 415 km) in the distal Bengal Fan from the present studies serves as a vital missing link between the Early Cretaceous and Late Cretaceous crusts evolved during the rifting and drifting of the Indian plate from the Antarctica–Australian plate during the Early Cretaceous period. Further, the present studies provided an observational

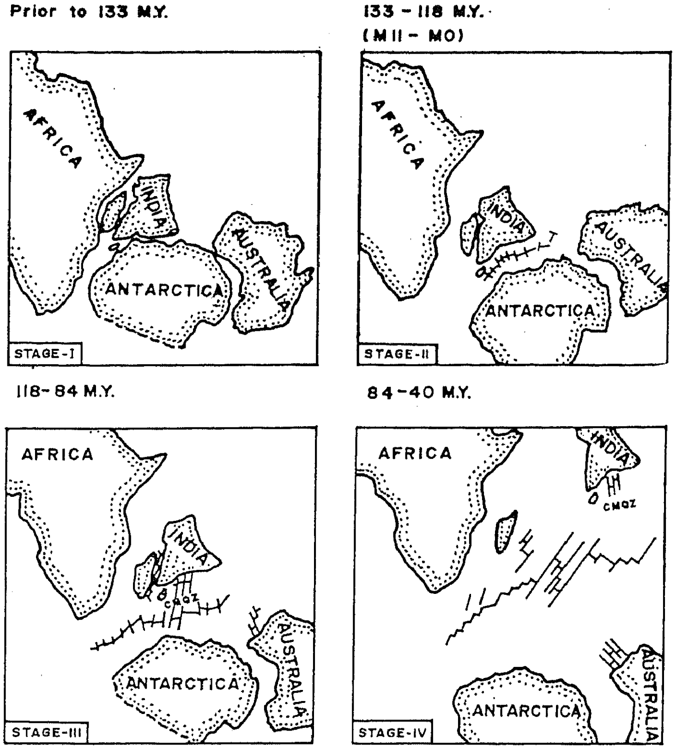

Figure 3. Schematic representation of the breakup of eastern Gondwanaland mass since the Early Cretaceous. *Stage I.* The configuration of the continents prior to 133 Myr; *Stage II.* India separated and moved in the NW–SE direction from the Enderby Landmass of Antarctica and about 1100 km wide Early Cretaceous crust, represented by the Mesozoic anomaly lineations M11 to M0, was evolved with an average spreading rate of 3.5 cm/yr. The spreading centre was between India and Antarctica; *Stage III.* The spreading rates decreased to 1.2 cm/yr and about 415 km wide Cretaceous magnetic quiet zone was evolved and India started moving from NW to NS direction; *Stage IV.* The northward movement of India was rapid and the late Cretaceous to Late Eocene crust with various tectonic elements like fracture zones, plateaus, seamounts and extinct spreading centres was evolved in the Indian Ocean. The evolutionary history of the northeastern Indian Ocean was well-documented since the Late Cretaceous to the Present.

evidence to develop a realistic plate reconstruction model of the eastern Gondwanaland after the Early Cretaceous breakup. We propose the evolutionary history of the Bay of Bengal in four stages (Figure 3) on the basis of the identification of the CMQZ, and the Mesozoic anomaly sequence M11–M0 and N120°E trending fracture zones[23,24]. During the first stage, the breakup of the Greater India from the contiguous Antarctica–Australian plate was initiated prior to 133 Myr. During the second stage (132.5–118 Myr) India separated from the Enderby Landmass of Antarctica and moved approximately in NW–SE direction, producing about 1100 km of Early Cretaceous crust characterized by M11–M0 anomalies in the Northern Bengal Fan. Around this time the continental extension between Antarctica and Australia took place and the eastern part of Greater India and Australia got separated, producing M10 and younger anomalies on the western margin of Australia[6]. In stage three (118–84 Myr), the direction of the Indian plate changed from NW–SE to N–S, with a slow spreading rate of about 1.2 cm/yr with spreading centre in between Indian and Antarctica–Australian plates and created about 415 km wide CMQZ. During this Cretaceous quiet period the trace of the Ninetyeast Ridge was also evolved[33] in the Bay of Bengal as the Indian plate moved over the Kerguelen hotspot, and the India's separation from Madagascar took place. During stage four (84–40 Myr), India's northward motion accelerated and Late Cretaceous to Late Eocene ocean floor was evolved with various tectonic elements like N–S trending fracture zones, plateaus, seamounts, extinct spreading ridges, etc. in the north eastern Indian ocean.

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Precious metal association in the Jaduguda uranium ore deposit, Singhbhum Shear Zone, Bihar, and its significance

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Concentrations of Pt, Pd, Au and Ag in some ore samples and sulphide-rich flotation products from the Jaduguda uranium deposit in Singhbhum (Bihar) are reported. In all the samples analysed, Pd far predominates over Pt, and Ag over Au. The geochemical significance of these concentrations is discussed.

THAT the copper ores of Singhbhum Shear Zone, Bihar, contain significant trace quantities of valuable metals like Au, Ag, Te, Se, Bi, Co, Ni is well known[1]. Some of these metals, like Au, Ag, Te and Se are recovered from the anode slimes generated during electrolytic refining of copper in the smelter of Hindustan Copper Ltd at Ghatsila, Bihar. These metals are concentrated along with copper during flotation. The Mosabani ore is reported to contain an average of 0.25 ppm Au[1] and the Rakha copper ore 0.07 ppm (ref. 2). The occurrence of suspected osmiridium has been reported in the mineral separates from Mosabani[3]. However no data on the contents of PGE, Au and Ag in the different uranium ore deposits of the Singhbhum Shear Zone are available. Here we present some data on the Au, Ag, Pd and Pt contents in samples of uranium ore from Jaduguda and in the sulphide mineral-rich concentrates separated from the ore in the Jaduguda mill of Uranium Corporation of India Ltd.

The following six types of samples were analysed:

i) Two bulk samples drawn from two different levels of Jaduguda mine and one sample of run-of-mine ore from Narwapahar Mine;
ii) A Ni-rich grab sample from the 295 m level, and a Cu-rich grab sample from the 550 m level, both from the footwall lode in the Jaduguda mine;
iii) A number of samples drawn from the bulk sulphide float produced in the by-product recovery plant (JBRP) of Jaduguda mill;
iv) A sample of the heavy mineral fraction of the bulk sulphide float, obtained by tabling;
v) Two samples of copper concentrate produced in the JBRP; and
vi) Some test products of roasting and leaching of the bulk sulphide float.

Pt, Pd, Au and Ag contents of most of the samples have been analysed by ICP-MS, Model PR1, manufactured by Fisons Instruments Inc., UK, in the Geochemistry Laboratory of National Geophysical Research Institute, Hyderabad. The samples, ground to a state that passed through 230# BSS, were dissolved using aqua regia, bromine and hydrofluoric, perchloric and nitric acids. Rh at a concentration of 100 ng/ml was used as the internal standard, as the samples were found to contain negligible amounts of rhodium. The details of the analytical procedure are published elsewhere[4]. The precision of estimation is better than ± 10%. The Au contents of a few of the samples have been analysed by INAA in the Analytical Laboratory of AMD, while the Ag contents of some of the samples have been determined by AAS in the Ore Dressing Section of BARC. The precision of these analyses is not known, but as the values are in the same range as obtained in the ICP-MS, are believed to be good.

The results are given in Table 1. Where three or more samples of the same type have been analysed, the

Table 1. Some precious metal contents in samples of Jaduguda uranium ore (all values in ppm)

Sample	Pt	Pd	Au	Ag
Jaduguda ROM ore				
Lot I (0 m level)	0.12	1.54	0.064*	2.0@
Lot II (434 m level)	0.15	1.44	0.051*	2.0@
Lot III (Comp. May 93)	nd	nd	nd	2.4@
Mean	0.135	1.49	0.058	2.1
Jaduguda ore sample				
Ni-rich sample from 295 m level	0.08	3.51	0.13	2.14
Cu-rich sample from footwall side of FW Uranium lode at 555 m level	0.01	0.85	0.03	12.28
Narwapahar ROM ore sample	0.06	3.03	0.070*	3.0@
Bulk sulphide float from BRP, UCIL, Jaduguda				
1	nd	nd	1.25*	11.0@
2	0.15	6.60	1.56	15.94
3	0.10	7.06	1.79	11.25
4	0.10	6.60	2.40	12.99
5	0.10	6.06	3.68	22.27
6	0.08	4.76	4.58	11.71
7	0.04	5.67	1.68	8.11
8	0.06	5.68	0.38	10.83
9	0.06	5.17	1.34	81.91
Mean	0.083	5.95	2.18	13.01
Standard deviation	0.033	0.78	1.29	4.34
Table con. from bulk sulphide float	1.09	45.7	53.40	63.40
Copper concentrate from BRP, UCIL, Jaduguda				
1	0.10	3.17	2.81	13.58
2	0.14	4.76	2.26	17.20
3	nd	nd	2.70*	16.0@
Mean	0.12	4.0	2.59	15.59
Standard deviation	–	–	0.29	1.84
Processed samples of Jaduguda BSF				
Residue of sulphatizing roast-leach	0.13	6.08	2.80	11.58
Thiourea leach residue				
1	0.06	4.36	1.11	5.90
2	0.11	3.85	1.01	5.08

All analyses unless stated otherwise are by ICP-MS.
*Analysis by INAA by Analysis Group, A.M.D.
@Analysis by AAS by Analytical Chemistry Group, ODS, BARC.
nd = Not determined.

mean and standard deviation values are also given in Table 1.

Table 1 shows that the uranium ores of Jaduguda contain significant concentrations of Au, Ag and Pd, and that part of these are concentrated during flotation of sulphides. A typical bulk sulphide float (containing 9% Cu, 8% Ni, 3% Mo and 0.4% Co) analyses 2.2 ppm Au, 6 ppm Pd and 13 ppm Ag, and these values could be considerably concentrated by gravity concentration to 53, 46 and 63 ppm respectively. These are economically significant concentrations as by-products, but for the fact that the tonnage involved is very limited (about 1500 TPY of bulk sulphide float only). No data on the precious metal concentrations in the bulk sulphide floats from the copper ores of different parts of the Singhbhum shear zone are available, except that the copper concentrate from Mosabani is reported to analyse about 3 ppm Au[1] and average 50 ppb Pt (range 25–70)[5], and that from Rakha 2 ppm Au[2]. The present data, particularly the predominance of Pd over Au, underscores the necessity to analyse all the uranium and copper ores from the Singhbhum Shear Zone and the sulphide concentrates obtained from them for their precious metal concentrations.

An intensive ore microscopic study of heavy mineral fractions obtained from ground Jaduguda ore has shown the presence of distinct xenomorphic particles of native gold, and a group of PGE minerals tentatively identified as merenskyite–kotulskite [(Pd,Pt) $(Te,Bi)_2$–Pd $(Te, Bi)_{1-2}$] and potarite (Pd Hg)[6]. The former PGE mineral is in intimate intergrowth with a mineral resembling sopchite (Ag_4 Pd_3 Te_4). However, confirmation of the identity of these minerals is possible only after EPMA study. It is also known that 'invisible PGE' can occur within sulphide minerals[7]. Nickel minerals like pentlandite, gersdorffite, melonite and members of NiTe–$NiTe_2$ solid solution are the major carriers of PGE, especially Pd[8]. Besides common pentlandite, both gersdorffite and melonite of composition Ni_3Te_5 are reported from the Jaduguda ore[9,10].

An interesting feature of the concentration of these metals is the relative enrichment of Pd, Pt and Au in the nickel-rich part of the lode and of Ag in the Cu-rich part of the lode. The analysed nickel-rich sample (containing about 2% Ni) is practically free from copper, while the Cu-rich ore sample is impoverished in Ni. This is to be expected because in the Jaduguda deposit the nickel lode nearly coincides with the foot wall lode of uranium, while the copper lode extends to the foot wall side of the uranium lode into the Dhanjori volcanics[11]. This spatial distribution may be taken to indicate different geochemical sources for U, Ni and Cu; while there is little doubt that the Dhanjori volcanics is the source for the copper[12], U and Ni seem to be derived from the Singhbhum granite-greenstone complex (SGGC)[13,14], a typical Archean cratonic mass occurring to the south of the Singhbhum shear zone. The PGE and Au concentrations are also probably derived from the SGGC. Further support to this view comes from the fact that the footwall uranium lode in the Jaduguda mine is confined, to a greater part, to a prominent quartz-pebble conglomerate (QPC) horizon and that the QPC horizons occurring elsewhere in the Singhbhum craton contain significant concentrations of gold[15,16].

A second interesting feature is the greater preponderance of Pd over Pt, a feature not generally recorded from primary occurrences of PGE. This may indicate a komatiitic parentage of the source rocks, which characteristically exhibit low Pt/(Pt + Pd) (enrichment of palladium) and Cu/(Cu + Ni) (enrichment of nickel)[17], or may be due to remobilization and precipitation, as Pd is geologically more soluble and hence mobile[18,19].

Considering that the weight of the bulk sulphide float in the JBRP is of the order of 0.6 to 0.8% of the feed to the flotation circuit, the recovery of the precious metals is only partial, though they are concentrated in the sulphide float. The Au/((Au + Pt + Pd) ratio in the ore samples analysed is about 0.03–0.04, which is within the range observed in most of PGE-mineralized rocks[8]. This ratio in the bulk sulphide float is 0.2 to 0.5 and hence it appears that gold recovery is better compared to PGE.

Can the PGE, Au and Ag values be recovered from the sulphide floats? Studies are in progress in the Ore Dressing group of BARC on the feasibility of recovering the Cu, Ni, Co and Mo values in the bulk sulphide float by sulphatising roasting, followed by leaching. From Table 1 it appears that the precious metal values remain in the roast-leach residue after sulphatizing roasting. Thiourea has been found to be a good leachant for gold[20,21], and the sulphatizing roast-leach residue was subjected to thiourea leaching in the presence of excess ferric sulphate for 6 h at a temperature of 70°C. The assays of the residue of this leaching are included in Table 1. While nearly 60% of the gold and silver values are leached out, the leachability of Pt and Pd are about 35%. It may be possible to improve the leachability of all these metals by optimization of leaching parameters.

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India's Exclusive Economic Zone – Resources, Exploitation Management. S. Z. Qasim and G. S. Roonwal (eds). Omega Scientific Publishers, B-7, Lajpatnagar, New Delhi. 1996. Price: Rs 600. 248pp.

Under the UNCLOS-III, the coastal states have jurisdiction of the sea up to 200 nautical miles from the coast line, termed the Exclusive Economic Zone (EEZ). The resources within the EEZ of about 2.0149 million km^2 which is nearly 61 per cent of the Indian area, therefore, belong to us.

As India prepares to explore and utilize the living and non-living ocean resources, tap the energy potential and use the coastal zone for harbours, industrial activity and tourism, as a new frontier for food and wealth, it is essential that we understand and assess the different aspects of this important marine ecosystem. This would enable us to utilize the resources in a rational way without causing damage, so as to maintain a sustainable relationship between exploitation and ecobalance.

To be able to manage this zone, we must know the long-term and short-term environmental problems faced by the various sectors of the zone and the consequences associated with the exploitation of its resources. To discuss this problem, a National Seminar on Management and Policies for India's EEZ was organized by the Planning Commission and the University of Delhi with the support of the British Council at the NIO, Goa. This volume presents a selection of papers presented at the seminar.

Qasim in his introductory essay explains what lies ahead and suggests that management of the resources would require an understanding of the types of features and the resources they contain, the establishment of proper infrastructure, trained manpower and use of environment-friendly technologies. The coastal belt up to 100 m depth at present being extensively exploited for a variety of purposes, demands rational management schemes to control possible harmful effects of the activities.

There is an imperative need for (1) the conservation and protection of the biological diversity, (2) the management of endangered ecosystems such as the mangroves, coral reefs and declaring them as biosphere reserves, (3) an understanding of the nature and influence of the coastal upwelling leading to high fertility in certain areas which are the sites of our major fisheries, and (4) examining the possibilities of mariculture, consequences and control of anthropogenic disturbances such as dredging and reclamation. There is also the need for further legislation, review and monitoring of the sources of pollution and control of coastal erosion.

Ocean resources must be used for the benefit of mankind but they must be used responsibly and sustainably so that future generations do not pay for our misuse. Antony S. Laughton warns that fresh consideration be given to using the ocean for properly engineered waste disposal and storage, with safeguards against unacceptable environmental degradation. Oceanic processes must be understood sufficiently to allow reliable models to be adopted to predict the state of the ocean and its reaction to perturbations. Laughton, therefore, recommends a continuous observing system to monitor the health of the oceans.

Considering India's industrial and economic development, there will be increasing demand for metals, such as copper, nickel, cobalt and silver as well as gold and platinum. Marine mineral resources and their sustainable exploitation are discussed in three papers by Roonwal, Chandra *et al.* and Victor Rajamanikom. The mineral potential of India's EEZ includes well-known monazite–ilminite rutile placers on the west and east coasts, calcareous aggregates and sands in the shallow waters, phosphate mud and sediments. Similarly, the cobalt-rich manganese crusts in several locations, and the hydrothermal sulphide mineralization in the Andamans seem promising. Further, the sea water components and the hydrocarbon deposits in the off-shore areas are also important. A good beginning has been made in respect of the exploitation of beach and offshore placers, heavy mineral sands comprising ilminite rutile, zircon, monazite, sillimanite and garnet; lime-mud deposits, calcareous sediments, phosphate nodules, phosphatized rocks, ferro-manganese nodules and possibly diamond and gold. The utilization of remote sensing application would be beneficial for evaluating placers and to monitor the changes which might take place in the configuration of the beaches.

The biological resources are by far the most important from the point of view of protein food for the Indian people. Five papers deal with biological resources.

Benthos form the basis for energy flow to many species in the benthic system, the meiofauna serve as an important food source for higher trophic levels. The macrofauna contribute to the benthic energetics of estuaries which is reflected in the demersal fishery. For the interpretation of the ecology of tropical demersal fisheries, an estimation of the benthic organic carbon is the basic prerequisite. According to Ansari *et al.*, the dimersal fisheries of the continental shelf have been assessed as 1.2 million tonnes as against the present level of exploitation of about 0.45 million tonnes per year. The nonavailability of base-line data on the benthos of the EEZ has been a serious constraint while assessing potential fishery resources. Wide variations exist in the distribution and faunal abundance, coastal waters and estuaries harbours high density. The average annual production range from 0.176 to 11.8 g cm^{-2} year. Benthic production is directly related to primary production.

Along the south-west coast, the overall productivity increases sharply during the SW monsoon on account of nutrient enrichment. High biomass of zooplankton in the region leads to large catches of fishes. The sardine and mackerel take advantage of the conditions that develop during the season. Their migration from south to north coincides with the south-north progression of the upwelling triggered by a shift in the availability of the food maxima.

The zooplankton is the chief index of utilization of the aquatic biotype at the secondary trophic level. The shelf areas sustain higher zooplankton production according to Goswamy, as compared to oceanic waters. Biomass values are higher over the shelf region during SW monsoon and in the oceanic realm during the premonsoon. Diel variability is reflected in the biomass – night values being higher than day values. On the basis of these the potential fish yield has been worked out as 9.01 and 2.25 million tonnes per year respectively for Arabian Sea, Bay of Bengal, Lakshadweep and Andaman Nicobar Seas.

Estimates of fishery potential based on the data of primary, secondary and benthic production for a period of over 20 years by Bhargava amount to 4.72×10^6 tonnes per annum. Since the annual production is just over 2 million tonnes it can be inferred that the production can be doubled.

Marine fishery is an established industry in India. Nearly 9 million people are involved. India is a leading sea food producer and exporter, the maximum export has always revolved around marine prawns fetching 80 per cent of the revenue. The present production has stabilized around 1.5 lakh tonnes annually. To increase the present production, deep sea prawns have to be located and trapped and aquaculture developed into a major industry. Achuthankutty suggests substantial increase in the present aquaculture production of 35,000 tonnes through expanding the activity and use of advanced technology.

In a power-deficit country such as India, renewable energy such as ocean thermal energy conversion, wave energy and tidal energy look promising and relevant. Ocean energy is pollution free and has minimum health hazards. According to P. M. Koola this resource is most valuable for island communities.

The volume also contains discussions on certain related topics. A set of recommendations are included at the end on developing coherent environment management policies for India's EEZ of which the most important is the formation of a high level committee in which the government, relevant institutions, universities and industry are represented to co-ordinate and monitor the management of the EEZ.

The present series of papers contain authoritative information on the resources by experts and these will draw the attention of researchers, managers and planners to the problems involved in the economic use of India's EEZ. The recommendations would be beneficial for evolving suitable strategies for the sustainable realization and management of the resources.

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Annual Review of Nutrition 1996. Donald B. McCormick (ed.). Annual Reviews Inc., 4139, El Camino Way, P. O. Box 10139, Palo Alto, California, 94303–0139, 557pp. Price: USA $53, elsewhere $ 58.

Annual Review of Nutrition is a valuable series that gives advances in both the basic and the applied aspects of nutrition. It is this blend that makes this publication useful to the research scientists, the teachers, and the practitioners of nutrition. Even science administrators and policy makers may find information that they can use.

The prefatory chapter 'On the making of a clinical nutritionist', is by Bob Olson — a man who has contributed significantly to the basic science and application of nutrition. This autobiographical account is interesting and informative, with an element of nostalgia regarding the events that led to some important discoveries in nutrition and biochemistry.

The overall emphasis of the present volume is on recent advances in nutrient transport mechanisms, regulation of metabolism and diet and disease, particularly cardiovascular disease. Not a single article deals with nutrient deficiencies, which however, continue to plague the developing countries, and about which a lot is being said in other forums. Advances in micronutrients such as vitamins A, E and folic acid are, however, covered in other ways.

A review by Butterworth and Bendich discusses prevention of birth defects (neural tube defects – NTD) by periconception supplementation of women with folic acid. The incidence of NTD is 5 per 1000 births. Though folic acid deficiency is widespread among Indian women, how much of NTD can be prevented by folate supplementation is a moot point since both genetic and environmental factors are responsible for its causation.

Vitamin E deficiency in humans is rare and generally secondary to malabsorption or metabolic abnormalities in lipoprotein metabolism, rather than dietary inadequacy. However, being a powerful antioxidant, vitamin E supplements are being promoted as prophylaxis against degenerative diseases where free-radical-mediated cell and tissue damage is implicated. Do epidemiological studies support this notion? How much vitamin E is enough? Traber and Sies discuss these aspects in the chapter 'Vitamin E in human disease and delivery'.

Besides vitamin E, there are other nutrients and phytochemicals in food (vitamin C, carotenoids, flavonoids, etc.) which have antioxidant properties. Their functions are reviewed in the chapter 'Antioxidants in human health and disease by B. Halliwell'. A lucid explanation of the mechanism of action of reactive oxygen and nitrogen species is also given. Oxidation of low-density lipoproteins (LDL) by cells in the artery wall is believed to initiate the complex chain of atherogenic events, in which other lipid constituents, especially cholesterol also play a role. The role of dietary modifications, with special reference to anti-oxidants and fatty acids in atherogenesis is discussed by Reaven and Witztum.

While polyunsaturated fatty acids (PUFA) have a cholesterol-lowering effect, they tend to increase LDL oxidation. The solution is – a PUFA-rich diet along with anti-oxidant supplements. An interesting recent finding is, mono-unsaturated fatty acids like oleic acid (present in olive oil) is as good or better than PUFA, and that may be the secret of lower incidence of CVD in mediterranean region. The PUFA of fish oil is also very good.

The question of safe limits of dietary cholesterol and ways of achieving it, is still controversial, and covered in the chapter 'Cholesterol policy and the primary prevention of coronary disease' by Naylor and Paterson. New molecular markers will provide better insights into lipid-related atherosclerotic risk.

Obesity is an important predisposing factor in CVD. Frequency of obesity is greater among high-fat than low-fat consumers. Why? For answers, read the chapter 'Control of human appetite: Implications for the intake of dietary fat' by Blundell *et al.* High-fat diets seem to have lower long duration satiety effect, than carbohydrates or proteins, the latter being the best.

Humans and animals have the enzymes for *de novo* lipogenesis. For long this has been regarded to be the method of storing surplus non-fat energy. However, recent studies using stable isotopes question the relevance of this pathway in humans raising several questions. The basis of this dilemma is discussed by Hellerstein in the chapter 'Regulation of hepatic *de novo* lipogenesis in humans'. Use of stable isotopes has greatly increased our understanding of the regulation of carbohydrate and fat metabolism during and after exercise, though questions persist as seen from the update by Halloszy and Kohrt.

The molecular mechanisms involved in transport of nutrients between tissues, their uptake by the cells and in some cases binding to DNA, through receptors, are beginning to be understood in recent years, thanks to the molecular biology tools. This volume of *Annual Review of Nutrition* devotes 5 chapters to these aspects. The role of peptide transporters, pept 1 and pept 2 in the intestine and the kidney has been discussed by Leibach and Ganapathy. Both types of receptors have been cloned and found to transport di- and tri-peptides using a transmembrane electrochemical H^+ gradient. They have pharmacological importance in the transport of peptide-like drugs. A family of transport proteins for glucose and fructose have been identified. They have different properties and tissue distribution. (Olson and Pessin), and are involved in the regulation of blood and tissue glucose.

A complex set of cytoplasmic, and nuclear proteins for vitamin A and its active metabolite retinoic acid have been identified. A recent hypothesis that the cytoplasmic proteins are involved in retinoid metabolism is discussed by Li and Norris in the chapter 'Structure/function of cytopolasmic vitamin A binding proteins'. Identification of retinoids which stimulate selective responses through nuclear receptors has helped to elucidate the molecular mechanisms involved. Besides these, synthetic agents also have therapeutic effects according to Pfahl and Chytil.

A. C. Antony's article on 'Folate receptors', discusses the structure, functions and regulation of folate receptors. These receptors bind folate and anti-folate compounds. Folate receptors have been exploited for uptake of toxic proteins or drugs (packed in liposomes and conjugated to folate), by target cells. This is a new approach for targeting drugs to cancer cells. Folate receptors also play a crucial role in transplacental transport of folate.

The 16th volume also includes a variety of other topics such as, mechanism of food allergy, effects of food on drug absorption, dietary change and traditional food systems of indigenous people, parenteral nutrition in low-birth-weight infants, and some more. It is indeed a mixed and interesting fare with something for anybody interested in food, nutrition and health.

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Annual Review of Biochemistry 1996. Charles C. Richardson. Annual Reviews Inc., 4139 El Camino Way, Palo Alto, California 94303–0139, USA. Vol. 65. Price: USA $59, elsewhere $65.

Every biochemist eagerly looks forward to the *Annual Review of Biochemistry* not only for its exhaustive reviews in the area of one's interest but also as a possible source for the search of new research problems. The personalized account of research by well-known scientists in the prefatory chapter is also something one looks forward to. This volume of *Annual Review of Biochemistry* has 25 articles including the prefatory article by Daniel Koshland Jr, the former editor of *Science*. Interestingly, in 11 of the 24 reviews, there is some reference to disease and/or suggestions for improved therapies. While in some of the articles, there may be justification, in others the connection is less apparent! Generally, the *Annual Review of Biochemistry* covers a broad spectrum of areas and grouping them into categories is not easy. However, 11 of the articles are related to biology of nucleic acids. The other topics cover enzyme biochemistry, various aspects of protein structure, cellular signalling and drug resistance in *M. tuberculosis.*

Aspects of DNA repair have been covered in four reviews. Friedberg and Sancar explore the biochemistry of DNA excision repair in detail in prokaryotes and eukaryotes with emphasis on repair mechanisms in humans and coupling of transcription and nucleotide excision. Defective excision repair in humans has been recognized to be associated with hereditary diseases xeroderma pigmentosum, cockayni's syndrome and trichthiodystrophy. Since the transcription factor TFIIH appears to have an obligatory role in nucleotide excision repair, newer approaches can be adopted to study the molecular basis of these hereditary diseases. How mismatch-directed repair in *E. coli* takes place and the genes responsible for this are discussed by Modrich and Lahue. Since these reactions appear to be conserved during evolution, the reviewers suggest that inactivation of their homologous genes in mice and humans results in a large increase in spontaneous mutability and consequently predisposition to tumour development. In the review on DNA repair in eukaryotes by Wood, the emphasis is on how DNA glycosylases and endonucleases carry DNA repair. Other articles related to DNA biochemistry describe how DNA helicases unwind duplex DNA, the regulation of teleomere length which includes the biochemistry of the enzyme teleomerase and other telomere-binding proteins and an update on DNA topoisomerases. The connection between cellular senescence, aging, cancer and telomeres is discussed in some detail in the review on telomere length regulation and should be of interest to those working in the molecular biology of cell division and malignant transformation.

Three articles discuss the biochemistry of ribonucleic acids with specific emphasis on mRNA. Splicing of mammalian pre mRNA has been extensively investigated and has also been the subject of several reviews. It is becoming increasingly evident that like in eukaryotic transcription and DNA repair, several proteins are involved in the splicing reaction of mammalian mRNA. The review by Kramer focuses attention on the various factors having splicing activities. The article has a table which indicates structural motif in these proteins which should be useful to those interested in correlating structure with function in nucleic acid-binding proteins. mRNA decay in eukaryotic cells is highly specific. The factors that initiate mRNA decay are covered in a review by Jacobson and Peltz. Recording, i.e. re-reprogramming of translation is extensively discussed by Gesteland and Atkins.

The review on molecular mechanisms of drug resistances in *M. tuberculosis* by Blanchard is relevant as the appearance of multidrug-resistant strains poses serious health problems, particularly as only few drugs are effective therapeutic agents against this microorganism. The development of resistance even against fluoroquinolone class of antibiotics calls for new strategies to combat tuberculosis. One suggestion by the reviewer is combined administration of β-lactams and β-lactamase inhibitiors. Since a large number of β-lactams are characterized, this approach could be both exploring as well as cost-effective compared to *de novo* synthesis and clinical evaluation of new compounds.

Mammalian amino acid transport with emphasis on genes coding for the various transporters and their regulation is reviewed by Malandro and Kilberg. Characterization of transporter genes should help in understanding the molecular basis of the autosomal recessive genetic disease cystinurea which is characterized by high levels of urinary cystine and cationic amino acids leading to cystine stones and renal disease.

Biochemistry of the '21st amino acid' selenocysteine and the enzymology of selenocysteine-containing proteins is reviewed by Stadtman. Proteasome are essential components of ATP-driven proteolytic pathway in eukaryotic cells. They are also involved in the conversion of transcription factors NF–κB and NF–κB2 from inactive to active forms and antigen presentation. These aspects are reviewed by Coux, Tanaka and Goldberg. The physico-chemical factors that influence interaction of sugars with lectins are becoming clear with the determination of the X-ray structures of several lectin–carbohydrate complexes. These aspects are dealt with by Weis and Drickamer. The 3D structures of the primary subunit, TATA box-binding protein and the core promoter of the transcription factor 11D have helped in understanding its molecular architecture. These aspects and recent functions on the complex interactions of the basal transcription machinery with regulatory transcription factors, especially through co-activators have been reviewed in detail by Burley and Roeder. Various aspects of structure and function of connexons and connexins, i.e. proteins that form gap-junctions that aid in intercellular communication, electron transfer in proteins and hematopoietic receptor complexes are subject of reviews that deal with protein structure.

In the area of cellular signalling there are reviews on signal transduction in the early stages of development in *Dictyostelium* (Parent and Devreotes), lipo-chitooligosaccharide molecules as signal factors used by rhizobia (Dénarié, Debellé and Promé) and cross-talk between the nucleus and the mitochondrion that enable assembly and proper functioning of proteins in the mitochondria (Poyton and McEwen).

Protein prenylation and protein transport across the endoplasmic recticulum and bacterial inner membranes have been popular topics for reviews and essays in recent years. Hence, it is somewhat surprising to find reviews on these two areas. While almost all the reviews are topical and exhaustive, four articles related to DNA repair are perhaps unnecessary in one volume.

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Deterministic Chaos: Complex Chance Out of Simple Necessity. N. Kumar. University Press, 3-6-272, Himayatnagar, Hyderabad 500 029. 1996. pp. 96 + xiii. Rs 70.

About a decade ago in *The New Physics* (ed. Paul Davies), Joseph Ford wrote a chapter entitled, 'What is chaos, that we should be mindful of it?*' . The asterisk at the end is for a footnote informing us that this is in fact a quote from Psalms in the *Bible.* The chapter begins with a quotation from *Hamlet*, 'There are more things in Heaven and earth, Horatio/Than are dreamt of in our philosophy'. The subject of chaos has truly linked together such diverse aspects of human intellecual existence, from spirituality to aesthetics to . . . well, the hard core determinism of classical physics. Deterministic equations governing the change in space and time of realistic natural objects are inevitably nonlinear. Chaos is a consequence of nonlinearity. No other branch of theoretical physical sciences has generated so much excitement in the last decade as chaos. Hoards of papers and a large number of books have appeared on this.

Spirituality and quotations aside, most books on chaos address Ford's question in colourful pictures of beautiful objects, real and abstract. In such a crowd of books on chaos, why another book? Well, for one, most of these fascinating books are nothing but library treasures and that too only available in a few libraries in a country like India. N. Kumar's book is a greatly needed monograph affordable for every college student.

But does it have everything that those books that are tens or hundreds of times more expensive have? Not really. But then, none of those books has everything you want to know about chaos either. James Gleick's popular book is nice to read once over, but it is too pedestrian for any serious student of science. Books with colourful pictures of turbulent smoke and fractal landscapes are attractive, but there is always something missing. Of course, books like the one by Georg Schuster are more comprehensive and certainly a necessity for most researchers in this area.

The JNCASR monographs are meant to be educational for students and teachers who may or may not be engaged in active research. This is where Kumar's concise and yet comprehensive monograph wins over the rest. It provides a reasonably comprehensive answer to Ford's question cited above.

Kumar starts the first chapter with a general overview, intentionally written in a discursive style. The second chapter elaborates the basic concepts like phase space, flows and Poincaré section, etc. Then come four chapters on details – simple models, strange attractors, conservative systems and fractals. A chapter on concluding remarks is followed by four appendices. These, though required as supplements to the text at different points, may be looked at right after the first chapter. Another appendix with a detailed analysis of an oscillating chemical reaction may have been useful.

There are a number of frontier problems judiciously left untouched except for brief comments in the concluding remarks. These include quantum chaos and controlling chaos. While these are important questions currently being pursued by active researchers, detailed discussion on these could not be made part of such a concise monograph with the specific purpose that it has – namely to supplement texts on nonlinear differential equations, classical physics, or statistical mechanics.

Kumar is one of the most well-known Indian physicists. The long span of his research career in classical physics and his experience as a physics teacher brings an authenticity to his rendering of the subject matter. In addition, he has a rare combination of smooth and precise diction. At times, however the necessary brevity makes the reading a bit condensed and for a beginner, it is likely to be difficult. For a graduate student, on the other hand, it should be a useful pocketbook to carry around. A major lacuna, and a surprising one from Kumar, who is known to indulge in problem-solving with any student who approaches him, is the absence of exercise problems. This is compensated by occasional provocations to carry out the various cases investigated in the text. This can be said about the discussions on the logistic map (the famous model used by Robert May for the measles epidemic) and baker's transformation, for instance. A number of good books are referred to now and then.

This and the other monographs published in this series are not yet widely known to most students and teachers in the country. Greater effort in publicising these books will be useful. A suggestion from this reviewer for a future monograph is for one on geometry and topology for students of physics and chemistry. This could be a complementary reading to a book on chaos.

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Errata

Plant life under extreme environments

H. Y. Mohan Ram
Curr. Sci., 1997, **72**, 306–315

The cover caption on page 290 should read: The vegetation in deserts is scanty. Goats have learnt to feed on whatever is available to sustain them (H. Y. Mohan Ram).

Page 310. Right-hand side, 2nd Figure, the caption should read: Sewan grass (*Lasiurus sindicus*) growing on sand. It is one of the most productive grasses of the Thar desert (M. M. Bhandari).

Page 311. In legend to the figure on left top, the name of the plant should read: *Suaeda fruticosa* and not *Suade fruticosa*.

Science policy in neo-liberal India: Corporate culture, basic science and scientific credibility

Suresh K. Mahajan
Curr. Sci., 1997, **72**, 295–299

Footnote on page 295: read 25 January instead of 24 January; read Dept of Civics and Politics instead of Dept of Civics and History.

Page 298, middle column, line 11 from top: read Rs 14,000 crores instead of Rs 1,400 crores.

Manganese mobilization from the Western Continental margin of India

D. N. Yadav
Curr. Sci., 1996, **71**, 900–905

Page 903, Table 2, column 2, 1st line. Number should read as ~ 21 instead of ~ 35.

Page 904, Figure 4. All units given for F_r, F_s, F_{Mn} should read as g/y instead of μg/y.

Page 905, 2nd line. Units for F_s, F_{Mn} should read as g/y.

Conformation of ATPMg(II) bound at the specific site on bovine serum albumin: ^{1}H-nuclear magmetic resonance study

Hari Pada Maity and Gotam K. Jarori
Curr. Sci., 1996, **71**, 906–914

1. Equation (5),

$$W_{ii}=\frac{\gamma^4\hbar^2\tau_c}{10}\left[1+\frac{3}{1+\omega^2\tau_c^2}\ \frac{6}{1+4\omega^2\tau_c^2}\right]\sum_{k\neq i} r_{ik}^{-6} \qquad (5)$$

should be

$$W_{ii}=\frac{\gamma^4\hbar^2\tau_c}{10}\left[1+\frac{3}{1+\omega^2\tau_c^2}+\frac{6}{1+4\omega^2\tau_c^2}\right]\sum_{k\neq i} r_{ik}^{-6} \qquad (5)$$

2. In Table 2

$$\tau=\frac{\nu_2}{\cos}\,p \quad \textit{should be} \quad \tau=\frac{\nu_2}{\cos p}$$

3. In page 909, under '*Binding of ATPMg(II)*'

$$|\omega_A+\omega_B|=\Delta\omega_M<\tau_A^{-1},\tau_B^{-1}$$

should be

$$|\omega_A-\omega_B|=\Delta\omega_M<\tau_A^{-1},\tau_B^{-1}$$

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MEETINGS/SYMPOSIA/SEMINARS

National Workshop on Fundamental and Analytical Aspects of Self-Organizing System

Date: 4–6 August 1997
Place: Raipur

Topics include: Fundamental of colloids, micelles, microemulsions, biomembrane, nanoparticles, surfaces and interfaces, industrial applications of surfactants, analytical methods and environmental effects, micellar catalysis.

Contact: Dr Kallol K. Ghosh
Organizing Secretary,
School of Studies in Chemistry
Pt. Ravishankar Shukla University
Raipur 492 010

Training Course in Basic Techniques in Animal Tissue and Cell Culture

Date: 18 August to 3 September 1997
Place: Pune

National Centre for Cell Science, an autonomous institute of the Department of Biotechnology, Government of India, is organizing a short-term training course in the above field. The course is limited to 20 mid-career scientists employed permanently and below 40 years of age. Depending on the response from the permanent staff, few seats may be available for JRF/SRF/RA. There is no course fee and the candidates will have to bear the lodging and boarding expenditure. However, accommodation may be available at the prevailing rate. Dead line for receiving duly filled in application is 15 June 1997.

Contact: Dr R. R. Bhonde/Dr K. S. Nnanda Kumar
Course Coordinators,
National Centre for Cell Science
NCCS Complex, Ganeshkhind
Pune 411 007
Phone: 0212–370951/961
Fax: 372259
E-mail: root@nccs.ernet.in

VII Workshop on Neutrons as Probes of Condensed Matter

Date: 7–25 July 1997
Place: Mumbai

Topics include: Theoretical aspects of neutron scattering, Neutron sources, detectors and instrumentation, Neutron diffraction (powder, single crystal, liquids and glasses), Magnetic neutron diffraction (polarized and unpolarized), Small angle neutron scattering, Inelastic and quasielastic neutron scattering, Neutron reflectometry, Data analysis procedures of neutron scattering data, Neutron interferometry and depth profiling.

Contact: Shri S. Mitra
Solid State Physics Division
Bhabha Atomic Research Centre
Trombay, Mumbai 400 085
Phone: 022–5563060 Extn 4264
Fax: 022–5560750
E-mail: iuc.@magnum.barc.ernet.in.

Fourth Training Course in Glaciology

Date: July–August 1997
Place: Chaurabari

The Geological Survey of India Training Institute, Hyderabad under the aegis of Department of Science & Technology will be organizing this Training Course. The course consists of two components with theoretical lectures at Lucknow followed by field demonstration/training on the Chaurabari Glacier to train the scientists/researchers having post-graduate degree in Geology, Meteorology, Physics, Geophysics, Hydrology with background in mathematics or a master/bachelor's degree in Mechanical/Civil Engineering and equivalent degree holder.

Contact: Dy.Director-General
Geological Survey of India Training Institute
GSI Complex, Bandlaguda
Hyderabad 500 068
Phone: 040–4020859, 4022681
Fax: 040–4022680

International Symposium on Free Radicals in Medicine and Biology

Date: 22–24 September 1997
Place: Udaipur, India

Contact: Prof. M. K. Barjatiya
Organizing Secretary, ISFRMB
Department of Biochemistry
R. N. T. Medical College
Udaipur 313 001, India
Phone: 91–294–528811–19 Extn 3124
Fax: 91–294–526383

International Workshop on Molecular Biology of Stress Responses

Date: 14–17 October 1997
Place: Varanasi, India

Topics include: Molecular biological aspects of thermal, oxidative, osmotic and starvation stresses.

Contact: Prof. S. C. Lakhotia
Organizer, IWMBSR
Cytogenetics Laboratory
Department of Zoology
Banaras Hindu University
Varanasi 221 005, India
Phone: 0542–316145; Fax: 0542–317457, 317074
E-mail: lakhotia@banaras.ernet.in.

CROSS DISCIPLINARY PLASMA SCIENCE PROGRAMME

The plasma state of matter abounds in many unique and exotic properties, nonequilibrium energy distributions, high energy density, intense charged particle and radiation flux, instabilities, self-organization, etc. This has contributed to its emergence as a powerful tool in a variety of scientific disciplines. Material synthesis and modification medicated by plasmas, non-equilibrium plasma chemistry, collective particle acceleration techniques, coherent radiation sources, etc. are successful examples of such cross-displinary linkage.

The Science and Engineering Research Council (SERC) of the Department of Science and Technology (DST), on a new initiative, has identified Cross Disciplinary Plasma Science (CDPS) as an area of thrust. Research programmes linking plasma physics with other branches of physics, material sciences, chemistry, engineering and environmental sciences will be identified and funded under the programme. The focus will be on basic scientific aspects, applications and technologies. SERC's ultimate objective is to identify and provide financial support for a number of well-motivated interdisciplinary projects.

Examples of CDPS areas are: Plasma based particle acceleration – Coherent radiation sources – Gaseous electronics for lasers – Microplasma displays – Plasma medicated surface sciences and engineering – Plasma chemistry – Pyrometallurgy – Synthesis of materials – Nanotechnology – Plasma techniques in environmental remediation – Isotope separation – Analytical techniques using microplasmas – Antennae in plasmas – Thermic energy conversion – Plasma diagnostics – Intense ion and electron beams. They are, by no means exclusive, creative search for extending these areas will be encouraged.

CDPS programme will be implemented under the following action plan: research opportunity reports will be prepared by participating experts in emergent and topical areas where plasma techniques and phenomena can make path-breaking contributions. These reports will be discussed in target group meetings and focused research projects will be generated. The proposals will be reviewed through a peer review mechanism and submitted for approval. The approval programmes will be funded by SERC and monitored through a Programme Advisory Committee.

Scientists and engineers from all disciplines from universities, educational and research institutions, interested in knowing more about and participating in the CDPS programme are invited to write to the following address, indicating their areas of interest.

Prof. P. I. John
Institute for Plasma Research,
Near Indira Bridge, Bhat Village,
Dist. Gandhinagar 382 428
Gujarat, India
Phone: 079–7869631/7864690/7864023
Fax: 079–7864310
E-mail: john@plasma.ernet.in.

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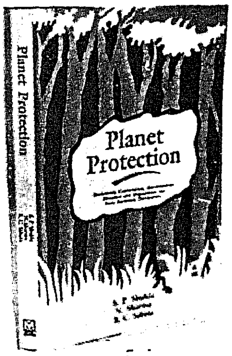

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The Government of Orissa, Department of Science and Technology has instituted one Samanta Chandra Sekhar (SCS) Award to be given each year for outstanding research contributions, during the five years preceding the award year by Oriya Scientists, working outside the State of Orissa, in any area of (1) Physical Sciences, (2) Life Sciences, (3) Engineering and Technology. Persons to be eligible for consideration for this award (a) should be Oriya by birth or domicile, (b) should have passed at least one of the following examinations from any recognised Institution/Board/University in Orissa.

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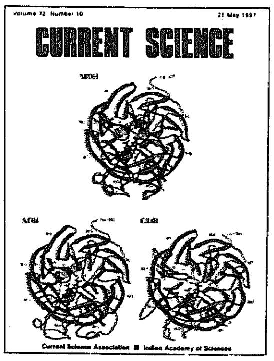

COVER. Schematic representation of the backbone of glucose dehydrogenase, methanol dehydrogenase and alcohol dehydrogenase. See page 716.

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Counting citations

Citation counting has become a popular pastime in the more advanced of our scientific institutions. Thanks to the availability of the Science Citation Index (SCI) in CD-ROM versions, the growing net of 'informatics' centres and a general inability to develop robust procedures for evaluation of science and scientists, 'citation analysis' is now increasingly used as a quantitative index for assessing scientific performance. Many government agencies have encouraged the uncritical use of SCI and journal impact factors as indicators of scientific quality. Not surprisingly, impact factors are uppermost in the minds of many publishing scientists. The paucity of citations to even good and relevant work emanating from India is often the subject of discussion, as is the limited contribution of Indian scientists to 'high impact journals'. It is truly amazing that a highly successful commercial organization in Philadelphia, the Institute of Scientific Information, should have such an overwhelming influence on scientists and managers of science. Are the results of citation analyses useful and meaningful? Is there science beyond the citation index? Száva-Kováts raises the bogey of non-indexed citedness (**page 705**). While the failure to consider eponymal references (no one, I suspect, refers to C. V. Raman's original papers when talking about the Raman effect) may cause less harm, given the wide impact of the work, there may be a greater problem in neglecting many sources not indexed by SCI. Ironically, in stating that 'non-indexed eponymal citedness' (NIEC) is a special case of Garfield's well-known 'uncitedness', the author fails to cite Eugene Garfield. To the many publishing scientists, who worry that diminishing citation counts may put careers in jeopardy, the author's conclusion – 'the meaning and value of quantified citation data of the Citation Indexes must be reduced, especially in the cases of the (mis) use of the data for the purpose of evaluating scientists as scientists' – must be comforting.

Quinoproteins

The tongue twisting pyrrolo-quinoline quinone (PQQ) prosthetic group was first identified in the mid-1960s in the enzymes methanol dehydrogenase and glucose dehydrogenase. The structure of the organic cofactor was established in 1979 and more recently, detailed three-dimensional structures of quinoproteins have been determined. C. Anthony and M. Ghosh (**page 716**) review the wealth of structural and mechanistic information available on PQQ-containing quinoproteins. A great deal remains to be clarified, particularly in understanding the precise routes and mechanism of electron transfer. As with most protein structures, there are always some surprises. In the case of methanol dehydrogenase, the stereochemical purists will be aghast to see two adjacent cysteine residues linked by a disulphide bond, with the intervening peptide unit deviating from planarity by 45°. Even Pauling's dictum that peptide bonds must be planar (or nearly so) can be violated.

P. Balaram

Transgenic plants

Improvement of plants using the sexual and asexual means has had a long history. Usually the transfer of genetic information is dictated by taxonomic barriers. Trespassing results in incompatibility, sterility or similar problems. Transgenic technology hinges on the ability to construct novel gene combinations and deliver them across taxonomic barriers. The communication by Sane *et al.* (**page 741**) is a case study.

The engineered gene has a 35S promoter from the cauliflower mosaic virus (CaMV 35S), the nopaline terminator (NOS) and cowpea trypsin inhibitor (*CpTI*) gene. The gene construct has been engineered into a plasmid of *Agrobacterium* and transformation effected by co-cultivation. *NPTII* has been used as a repeater gene in the study.

These protocols developed in an 'ideal plant – tobacco' can be used in engineering crop plants at a later date. The whole idea is to design genes with the ideal combination of constituent 'parts' borrowed from different organisms if required. The gene construct should be able, when incorporated into the plant, to produce the gene product at the right time, in the right tissue, in the desired quantity and in response to the external stimuli.

Sane *et al.* have transformed tobacco and incorporated insect resistance, shown the effectiveness of the gene by feeding trials. The segregation of the *NPTII* gene has been followed for a few generations in a stable manner. It will be interesting to see the segregation pattern of the *CpTI*, the candidate gene at a later stage.

H. E. Shashidhar

Current Science

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CURRENT SCIENCE

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CORRESPONDENCE

Funding research projects: We need to be wiser

It is time that the government agencies reconsider their policy regarding funding research projects in different universities and institutes. The agencies should consider not only the quality of the proposed research plan and the infrastructure available, but also the academic environment of the respective university/institute where the work would be carried out. Even if the proposed plan is significant and the person concerned is competent enough to carry it out, if the academic environment of that particular institution is not healthy enough, the proposed plan is almost bound to yield no result, and so is unworthy of funding. Also, the evaluation of the progress of the work should be more critical, considering the output of the previous work which can be judged only from the quality of the publications, and *not* by the number of publications.

For example Banaras Hindu University, a premier academic institute of the country, is also home to many non-academic elements who have their own political ambition, in pursuit of which they create all kinds of problems every year. The net result is, we lose precious time. Undergraduate/post-graduate students suffer because they are frequently called out of classes by these undesirable elements and their followers. Research suffers, because one just fears working under such conditions and gradually motivation diminishes. Even though the University administration has its own force of proctors, they are utterly helpless under these circumstances. Every second or third year there is *sine die* closure for an indefinite period. The University administration seems absolutely unwise in tackling these situations (and it may be helpless too). When there is a severe problem, even though the Administration knows the key figures behind it, it fails to take action against them. Instead, it adopts a rather easier (but suicidal!) way to *solve* it; it declares indefinite closure. And the closure can go for months! Undergraduate/postgraduate students are then forced to take their exams without covering the syllabus. And sometime, like the *sine die* closure of 1997, the administration can even be more imprudent. It feels that perhaps the easiest way of solving these problems is to close the research activities for an absurd length of time. It is true as daylight that behind many of the disturbances in BHU, invariably a number of research students are found to be involved. But the University administration cannot take individual action against them, because they receive backing from political parties. So the easy way is, to condemn hundreds of researchers!

Since the University administration feels that research activities can be stopped for any length of time, the question can rightly be raised as to why funding agencies should support research projects in such institutions? The question should be even more relevant to the UGC, as many of the departments in this particular university are regarded as Centres of Advanced Study and thus they receive substantial grants from the UGC. The UGC as well as other funding bodies should not fund an institution where the very basic requirement for research, an academic environment, is at stake. I do not say that the country should stop its research activities, but certainly it should do so in places proved to be unworthy of support. We are not only wasting public money, but are also condoning a Himalayan wastage of intellect. And this crime should continue no further.

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Discussion meeting on instability and transition in fluid flows – A report

A discussion meeting on instability and transition in fluid flows was organised by R. Narasimha under the auspices of the Indian Academy of Sciences, during 22–25 November 1996. About twenty-five participants gathered at Orange County resort, in the idyllic setting of Coorg, characterized by its hilly terrain, deep green forests and plantations, and located near the banks of the river Cauvery. The sessions were designed so as to have invited lectures by many of the participants, followed by discussion and question/answer sessions after each talk.

The meeting was mainly intended to get together Indian researchers working in the field of instability and transition. Michael Gaster and Jitesh S. B. Gajjar, both from the United Kingdom and at that time academic visitors at the Jawaharlal Nehru Centre for Advanced Scientific Research and the Indian Institute of Science, Bangalore, also participated in the meeting.

While the subject of Instability and Transition may seem very specialized, this is quite a broad area in the field of fluid mechanics, and it was not the objective of the meeting to concentrate on any focussed topic; rather, the objective was basically to look at each others' work, and thereafter to look at common themes or possible common future endeavours. Towards both these objectives the meeting proved to be a success, and was very valuable to the participants. It will therefore be best to report briefly on the talks that were presented, and later on to look at what seemed to emerge as possible common endeavours of enquiry.

Rama Govindarajan of National Aerospace Laboratories, Bangalore, spoke on 'Low order theories for stability of non-parallel boundary layer flows'. Essentially the objective of the work was to take a re-look at the full non-parallel equation for boundary layer flow, and decide on which were the most important (in some sense) terms that needed to be retained. The terms retained are the following. First, the Rayleigh operator; secondly, *only* the $(1/R_e)D^4\phi$ term from amongst the viscous group of terms in the classical Orr–Sommerfeld equation; and thirdly, in order to include the effects of non-parallelism, *only* the term $p\Phi D^3\phi$ from amongst the non-parallel group of terms. In the above, Φ and ϕ are respectively the mean-flow and perturbation streamfunctios $D = \mathrm{d}/\mathrm{d}y$ with y as the co-ordinate normal to the plate, R_e is the Reynolds number of flow, and p is a co-ordinate scaling factor. Notable amongst the terms rejected is the explicit $\partial\phi/\partial x$ term (x being the streamwise co-ordinate); and thus, the governing equation becomes an ordinary differential equation. The rationale for rejection of the various terms is based on two arguments. First there is an asymptotic justification for the terms rejected as these are of higher order than the terms retained. Second, the mean-flow would have to be known to a higher order if the additional terms were to be kept. Rama presented numerical results to support her claim, and the results did seem to support her contention. Sen pointed out that the rejection of the $\partial\phi/\partial x$ term was consistent with Sen's concept of optimal normalization.

P. K. Sen of the Indian Institute of Technology, Delhi, spoke on 'Non-classical solutions of the boundary layer stability problem', based on work done with M. D. Thomas. Sen reviewed the classical outer boundary conditions for this problem, viz. $\phi \sim \exp(-\alpha y)$, where ϕ is the disturbance streamfunction, α is the spatial wave number, and y is the co-ordinate normal to the plate. He went on to show the uniqueness of the classical solution, basing his arguments on the method of adjoints. Regarding non-classical solutions, his main contentions were as follows. In the event that an Orr–Sommerfeld like equation $L(\alpha)\phi = 0$ is augmented by an ε-order term, and the equation is modified to $L(\alpha)\phi = \varepsilon L_2\phi$, where L_2 is a linear operator; then, it is possible to obtain a non-classical 'secular like' solution in the following manner. First, expand ϕ as $\phi = \phi_e + \varepsilon\phi_n$, where ϕ_e satisfies $L(\alpha)\phi_e = 0$, and ϕ_n satisfies $L(\alpha)\phi_n = \varepsilon L_2\phi_e$. However the outer boundary condition for the equation for ϕ_n is the *secular like* condition $\phi_n \sim y\exp(-\alpha y)$. This composite solution for ϕ is the non-classical solution, since two parts of ϕ, viz. ϕ_e and ϕ_n, have different manners of decay in the outer region. The net outcome of the non-classical solution is that much higher growth-rates can be seen to exist so that the results are in much better agreement with experiments. Sen showed numerical results based on the non-classical solution to illustrate this point. Sen also mentioned that there was no contradiction with the uniqueness of solutions, because different eigenvalues were attributable to different mode-shapes. It so happens that the non-classical solution yields an eigenvalue with a higher growth rate.

J. S. B. Gajjar of Manchester University, UK, spoke on 'The stability of 3D boundary layer flows'. The flow configuration chosen was flow over a rotating disc. The problem was investigated using a combination of asymptotic and numerical methods for studying the linear and nonlinear stability properties of 3D incompressible/compressible boundary layers. Numerical results obtained for linear stability of the rotating disc problem, using a spectral collocation code, were shown to compare well with the earlier results of Balakumar and Malik. The absolute instability results of Lingwood were also verified. In addition, a near-neutral double zero was obtained in the dispersion relationship, indicating that algebraic growth may also be an important mechanism in the breakdown of the boundary layer over a disk.

Gajjar also described the nonlinear stability of cross-flow instability modes in incompressible/compressible boundary layers over a rotating disk, using multiple-scale techniques. The analysis leads to novel integro-partial differential equations for the vortex amplitudes. These equations have a number of interesting properties which may be of relevance to some experimental observations. These include nonlinear saturation, secondary instabilities as well as finite time/distance singularities which may lead to abrupt transition.

On the second day, M. Gaster of Queen Mary and Westfield College, London, spoke on 'Receptivity to free-stream disturbances'. The ultimate goal of transition-related research is to be able to predict the transition of the laminar boundary layer to a turbulent state. One aspect of the problem involves assessing, in a fully quantitative way, the magnitude of instability waves generated by free-stream turbulence. Turbulence in the free stream consists of vorticity fluctuations that convect past the plate (aerofoil) at free-stream velocity. There is, therefore, a mismatch in frequency–wave number spectra between the turbulence and the instability waves; the latter travel roughly at one-third the free-stream speed.

With the scope of the problem as above, Gaster's study was initially concerned with a number of simple boundary value problems. The calculations for perturbations in a steady boundary layer were carried out based on some simplifying assumptions: viz. linearization of the perturbation equations, reduced boundary conditions, together with neglect of boundary layer growth. The flows created by a suction slot and by a shallow bump were determined and compared with the measured flow patterns. The graphs displayed excellent agreement. Studies more recently performed considered 2D and 3D oscillating bumps. These problems were theoretically analysed, and the results compared well with measured data.

Regarding the problem of excitation by free-stream disturbances, the disturbances created by moving boundary value perturbations were calculated by similar methods. Gaster reported that when the boundary perturbation moved with the free-stream velocity, only disturbances related to the continuous spectrum are excited, and no travelling wave modes are generated. The other important point made was as follows. The boundary layer is not strictly a parallel flow, and *boundary layer growth enables the continuous spectrum to couple with the eigensolution.* This can be understood by treating the growing boundary layer as regions of parallel flow joined at a discontinuity. As the disturbance waves pass through the discontinuity, waves of all type are generated. The full receptivity problem would require the solution of the inhomogeneous disturbance equation, and this would need to be done in future.

T. K. Sengupta of the Indian Institute of Technology, Kanpur, investigated the flat-plate boundary layer subjected to very low frequency three-dimensional disturbances. The corresponding modes, called Klebanoff modes, are somewhat different from the classical Tollmien–Schlichting modes. The Klebanoff modes do not admit a viscous critical layer mechanism, because the αR_e-product is small (α is the streamwise spatial wave number and R_e is the flow Reynolds number), as α is very small. With the disturbance streamfunction and the distance normal to the plate given respectively as ϕ and y, it was seen that the ϕ_y distribution does not show a zero crossing. Therefore with the r.m.s. streamwise velocity fluctuation given as $u' = 2|\phi_y|$, the u' distribution does not depict two maxima as in the case of the Tollmien–Schlichting modes. For a fixed R_e and frequency ω_0, a spanwise wave number was chosen as $n\beta_0$, where β_0 is the spanwise cut-off wave number. Following this the eigenvalue(s) α were determined, first roughly by the method of Mack, and later polished by a Newton–Raphson technique. The stability calculations were done by the compound matrix method. Several interesting results were obtained and conclusions drawn thereform. (i) It was found that for these Klebanoff modes, $\alpha_r \sim O(10^{-4})$. (ii) The smallest α_i occurs for the 4th and 5th modes, and *not* for the first (fundamental) mode. (iii) Phase speeds are very small for the higher spanwise modes. (iv) The group velocity in the streamwise direction is about one hundred times larger than that in the spanwise direction. Thus there is streamwise energy transfer. (v) Higher spanwise modes have an eigenfunction compatible with experimental results. (vi) Newer streamwise modes appear as n increases, but these are not dominant. (vii) At very low frequencies (≤ 1Hz at $R_e = 1196$) the shear layer supports free stream excitation. (viii) The corresponding 2-D disturbances are upstream propagating, and continue to exist at higher frequencies.

V. Kumaran of the Indian Institute of Science, Bangalore, discussed 'Stability of fluid flow in a flexible tube'. The motivation for the work reported was that some past experiments of Krindel and Silberberg indicated that the transition Reynolds number in a flexible tube could be much lower than that in a rigid tube. The geometry chosen for investigation in Kumaran's work is an inner pipe of radius R, surrounded by an elastic wall of radius H, with $H > R$. The relevant parameters in the problem are the Reynolds number $R_e = \rho VR/\eta$, and another property $\Sigma = \rho ER^2/\eta^2$ that depends on properties of the wall material and the fluid, where η is the viscosity and E is the coefficient of elasticity. The equations of motion were set up for the fluid side along with the elasticity equations for displacements in the solid side. Appropriate matching conditions were used at the fluid solid interface. Thereafter, the perturbations were investigated using the usual normal mode analysis. The methods of solution used were a mixture of analytical and numerical methods. Results were presented for the viscous limit ($R \to 0$), inviscid limit ($R \to \infty$, $(\rho V^2/E) \sim 1$), and for wall modes for which $(\rho V^2/E) \sim R_e^{-1/3}$.

The main conclusions drawn for the viscous modes were as follows. Instability could occur for $\Gamma = (V\eta/ER)$ exceeding a critical value even for $R_e = 0$. Instability was due to shear work done at the interface. Results could be analytically continued to intermediate Reynolds numbers from the zero Reynolds number limit. Also there is a complex dependence of the critical Reynolds number on the ratio of viscosities of the fluid and the wall. The main conclusions drawn for the inviscid modes are as follows. Parabolic mean flow is stable to axisymmetric disturbances but could be unstable to non-axisymmetric disturbances, though both kinds could be unstable in the developing region of flow. Also, transport of energy due to shear work at interface is balanced by opposite transfer due to convection. And, dissipation in the wall has a stabilizing effect. The main conclusions drawn for the wall mode are as follows. Introducing the parameter $\Lambda = (E/\rho V^2)$, it is seen that the least stable solution does not converge to any of the rigid tube solutions, but has a frequency that diverges proportional to $\Lambda^{1/2}$ for $\Lambda >> 1$. By numerical analysis it is found that this mode becomes unstable when $\Lambda < \Lambda_c$ at fixed R_e, or when $R_e > R_{ec}$ at fixed E (subscript c depicting critical values). And, stability characteristics are similar to that of viscous modes for $R_e >> 1$.

J. H. Arakeri presented his work with D. Das on 'Transition of unsteady velocity profiles with reverse flow'. This is very interesting because it can be looked upon as either stability theory invoked to explain experiments, or as an experiment performed to verify some precepts of stability theory. The experimental set up (for pipe flow) consists of two straight and long lengths of a pipe connected at the ends in a loop. A piston motion in one of the pipes induces quasi time-dependent flow in the other pipe, which also contains the test section for visualization. The piston undergoes what is called 'trapezoidal' motion: viz. rapid acceleration from rest, followed respectively by a long period of constant velocity, rapid deceleration to rest, and a long period of rest. The velocity–time curve of the piston looks like a trapezium. During the long rest phase of the piston, i.e. at the last phase of the cycle, the flow through the second pipe (which contains the test-section) has reverse flow at the walls, and the velocity profile is inflectional. As time elapses, the effect of viscosity is to reduce the difference between the maximum and minimum velocity (since piston motion has already stopped) till the fluid comes to rest. The centre of attention is the period during which the velocity profile is inflectional. Interesting observations were made, and conclusions drawn, both based on visualization studies and by stability analysis. A video-film was also shown on the visualization experiments.

Visualization studies showed that the flow in the inflectional velocity profile becomes unstable with possibly a helical vortex forming. The wavelength of the instability is $\simeq 3.1 \bar{\delta}$ where $\bar{\delta}$ is the average boundary layer thickness, the average being taken over the time the flow is unstable. The time of formation of the vortices scales with the average convective time scale and is $\cong 37\delta / \Delta u$, where $\Delta u = (u_{\max} - u_{\min})$ and $u_{\max}$, $u_{\min}$ and δ are respectively the maximum velocity, minimum velocity and boundary layer thickness at each instant of time.

Quasi-steady linear stability analysis bring out two important points. First, that the stability characteristics of velocity profiles, with reverse flow near the wall, collapse when scaled with the above variables. Second, that the wave number corresponding to maximum growth does not change much during the instability phase even though the velocity profile changes substantially. Further, the instability is essentially inviscid and growth rates scale with convective instabilities. Even at large Reynolds numbers the viscous effects are unimportant because the velocity profiles change significantly during the period of instability. Further, the time to instability is $t = 28\delta/\Delta u$, and the time to vortex formation is $t = 37\delta/\Delta u$. It is also seen in the study that, despite instability occurring along with the associated vortex formation, transition to turbulence does not occur unless the Reynolds number based on boundary layer thickness exceeds some critical value. And, when transition does occur, it occurs very rapidly. An estimate shows that with the characteristic time to instability given as $t = 28\delta/\Delta u$, and with $\alpha = 2\pi/(3.1\delta)$ and $c_i = 0.15\Delta u$, the total amplification for wave formation is $e^{\alpha c_i t} = e^{8.5}$. Also, with characteristic time for vortex formation as $t = 37\delta/\Delta u$, the total amplification for vortex formation is $e^{11.2}$. These figures for amplification compare well with the e^9 growth typical of transition to turbulence. Thus many aspects of transition in oscillating pipe flow seem amenable to explanation based on visualization and stability studies. T. K. Vashist contributed to the development of the stability code.

D. Venkateswarlu of Vignana Jyothi Institute of Engineering and Technology, Hyderabad, spoke on his work with P. K. Sen on 'The stability of pipe-Poiseuille flow'. This is one of the few papers in the conference where nonlinear stability was discussed. The formulation of the problem was based on the Stuart–Watson methodology using the equilibrium amplitude assumption of Reynolds and Potter. The disturbance streamfunction is decomposed into a fundamental ϕ, and harmonics ϕ_n with $n \geq 2$. Further, each level of harmonic is in turn expanded in a series, in terms of powers of the amplitude A of the fundamental. The amplitude A in turn obeys the Landau equation, viz.

$$\mathrm{d}A / \mathrm{d}t = \alpha c_i A + \alpha A \Sigma_{n=1}^{\infty} k_n A^{2n} .$$

In the equilibrium amplitude formulation employed, $\mathrm{d}A/\mathrm{d}t$ is *ab initio* set equal to zero, in anticipation of equilibrium states, and the Landau constants k_n determined. Following this the equilibrium amplitudes A_e are determined. Since pipe flow exhibits no neutral curve to linear disturbances, the aim of the work was to see whether destabilization of the flow could take place in the presence of finite amplitude disturbances. The results for A_e were obtained by accelerated convergence methods using the method of Pade approximants. Finally, a curve for A_e versus the Reynolds number R_e depicted significantly that A_e decreased with R_e and was as low as around $A_e = 0.002$ at $R_e = 6000$. Thus it was concluded that finite amplitude disturbances could destabilize pipe flow. One notable feature of the work was the Noumerov transform molecule developed for $O(h^6)$ accuracy up to the fourth derivative in ϕ, and with a seven-point finite difference scheme.

T. K. Vashist of Centre for Airborne Systems Bangalore, spoke on 'Stability of compressible boundary layer flow'. He described the status of the work done so far by himself. The set of disturbance equations based on the linearized Navier–Stokes equations, continuity equation, energy equation, and the equation of state, results in an eighth order system in the disturbance variables u', v', ω', T', p', respectively depicting the three velocity components, and temperature and pressure. This is reduced to four ordinary, second-order, coupled differential equations by normal mode analysis. The boundary conditions at the edge of the boundary layer are the exponential decay conditions. At the wall, the velocities u', v', $\omega' = 0$. Also the temperature T', if expressed as overheat with respect to the wall, satisfies the condition $T' = 0$. However, the pressure term p', appearing only up to the first derivative, can satisfy only one boundary condition, and $p' \neq 0$ at the wall. The pressure term either needs a special condition to be derived at the wall, or it has to be eliminated altogether by cross differentiation. In addition, the mean flow and temperature fields need to be determined or prescribed. Vashist described a method for obtaining the mean flow for the two-dimensional case. He also described his numerical formulation for the eigenvalue problem. After the usual normal mode analysis in the form

$$\{u,' v', \omega', T', p'\} = \{f(y), g(y), h(y), \theta(y), P(y)\} \exp\{i(\alpha x + \beta z - \omega t)\},$$

the problem was first converted into finite difference form using a Noumerov transform developed for the purpose, such that the errors were $O(h^6)$ in all the derivatives (up to the second order) with only a five-point scheme. After concatenation of the matrix the non-zero terms were along the leading diagonal. Thereafter, the eigenvalues were sought to be determined first by the Thomas method, i.e. based on Gaussian elimination; and second, by the Q–Z algorithm. It is at this point that success is still awaited because the eigenvalues obtained so far seem not to tally with those existing in published literature. Further work is going on to make the programmes operational.

N. Rudraiah, of UGC-DSA Centre in Fluid Mechanics, Bangalore University, spoke on 'Linear and nonlinear Rayleigh–Taylor instability'. Rayleigh–Taylor instability occurs when a heavy fluid is supported by a lighter fluid in the following configurations:

(i) When there is a thin fluid layer supported by a rigid-plate below, and on top there is a densely packed porous layer with a heavy fluid.

(ii) When there is a thin film consisting of a fluid saturated porous layer, supported by a rigid plate below, and with a heavy fluid above.

Apparently these problems have not been investigated in spite of their importance in materials science, inertia-controlled fusion, astrophysics, geophysics, and biomechanics. The crux of the problem is to specify a proper boundary condition at the interface between the fluid and the porous medium. When the thickness of the porous layer, bounding the thin fluid above, is large, the slip boundary conditions that could be used are either the Beavers and Joseph condition or the Saffman condition. These boundary conditions are independent of the thickness of the layer, and hence valid only when the thickness of the porous layer is comparatively very large, as is the case in geophysical and some industrial problems. On the other hand, in many biomechanics problems the thickness of the fluid layer plays an important role; and, this led to a new boundary condition to be proposed by Rudraiah. This boundary condition is more suitable because it involves the film thickness. Using all the three conditions stated above, Rudraiah obtained the dispersion curve, and arrived at the following conclusions:

(i) The shape of the dispersion curve is mainly controlled by the surface tension and buoyancy forces, and the porous parameter and the thickness of the layer.

(ii) With suitable values of porous and slip parameters, both stable and unstable interfaces are found to exist.

A. Prabhu of the Indian Institute of Science, Bangalore, spoke on his work with J. Dey, R. Narasimha, M. Jahanmiri, S. V. S. Phanikumar and O. N. Ramesh, on 'Three dimensional spots'. This was a report on a series of experiments carried out in a distorted duct to study the effect of streamline curvature (transverse), followed by divergence with straight streamlines, on the structure of a turbulent spot. The experimental set up comprised a uniform duct, followed by a distorted duct that became uniformly narrower in the vertical direction and uniformly wider in the transverse direction. This ensured that there was no pressure gradient in the distorted duct since the average sectional velocity was maintained constant. Nevertheless, distortion of the duct caused the streamlines first to curve, and then to diverge (as straight streamlines), in the spanwise direction. The spot source, consisting of a pulse of air through a static hole on the test flat plate, was located in the uniform section upstream of the distortion. This spot spread and propagated downstream, and generally moved along the direction of the curved streamlines, followed by the diverging straight streamlines. Typical measurements were carried out at 0.5 mm above the bottom flat plate. It was found that the distortion produces substantial asymmetry in the spot. The angles at which the spot cuts across the local streamlines are altered dramatically (in contradiction of a hypothesis commonly made in transition zone modelling), and the Tollmien–Schlichting waves that accompany the wing-tips of the spot are much stronger on the outside of the bend than on the inside. However, there is no great effect on the inner structure of the spot and the eddies therein, or on such propagation characteristics as overall spread rate and the celerities of the leading and trailing edges. Both lateral streamline curvature and non-homogeneity of the laminar boundary layer into which the spot propagates, appear to be strong factors responsible for the observed asymmetry.

J. Dey of the Indian Institute of Science, Bangalore, presented his work with R. Narasimha on 'Momentum balance in the linear-combination integral-model for the transition zone'. The linear-combination integral method of Dhawan and Narasimha for transition zone modelling is one among various models now available. The momentum balance aspect of this integral model was taken up for discussion, in the presentation. In the Dhawan–Narasimha model the velocity u and the skin-friction coefficient C_f are expressed in composite form in terms of the laminar ('*L*'), turbulent ('*T*'), and composite ('lc') parts, and in terms of the intermittency γ. The expressions are

$$u = (1-\gamma)u_{\mathrm{L}} + \gamma u_{\mathrm{T}}; \text{ and,}$$
$$C_{\mathrm{flc}} = (1-\gamma)C_{\mathrm{fL}} + \gamma C_{\mathrm{fT}},$$
$$\gamma = 1 - \exp(-0.41\xi^2);\ \xi = (x - x_t)/\lambda$$
$$\lambda = x_{\gamma=0.75} - x_{\gamma=0.25},$$

where x is the streamwise co-ordinate. It was pointed out by Green that the method could lead to a momentum imbalance for constant pressure flow. To evaluate the error, the momentum thickness is expressed as follows:

$$\theta_{\mathrm{lc}} = (1-\gamma)\theta_{\mathrm{L}} + \gamma\theta_{\mathrm{T}} + \gamma(1-\gamma)\int(u_{\mathrm{L}} - u_{\mathrm{T}})^2\mathrm{d}y,$$

where θ is the momentum thickness. Thereafter, noting that

$$\mathrm{d}\theta_{\mathrm{L}}/\mathrm{d}x = 0.5C_{\mathrm{fL}} \text{ and } \mathrm{d}\theta_{\mathrm{T}}/\mathrm{d}x = 0.5C_{\mathrm{fT}},$$

one obtains

$$\mathrm{d}\theta_{\mathrm{lc}}/\mathrm{d}x - 0.5C_{\mathrm{flc}} = I_1 + \mathrm{d}I_2/\mathrm{d}x,$$

where

$$I_1 = (\theta_{\mathrm{T}} - \theta_{\mathrm{L}})\mathrm{d}\gamma/\mathrm{d}x, \text{ and}$$
$$I_2 = \gamma(1-\gamma)\int(u_{\mathrm{L}} - u_{\mathrm{T}})^2\mathrm{d}y.$$

Thus the error is given as $I_1 + \mathrm{d}I_2/\mathrm{d}x$. The present results were verified with respect to past experimental results on transitional boundary layers. The conclusion was that the momentum imbalance was amounting to about 2% in drag in the transition zone.

The next paper was by O. N. Ramesh, of the Indian Institute of Science, Bangalore, who presented his work with J. Dey and A. Prabhu on 'Transition zone in a constant pressure laterally diverging flow'. Experiments were conducted in a distorted duct having lateral divergence of streamlines under constant pressure (set-up already described in presentation by Prabhu), in order to study the effect of lateral strain rate on natural transition. Measurements showed that the normalized intermittency distribution follows the universal intermittency distribution of Narasimha. This is used to predict the transitional velocity profiles using a linear combination model which combines laminar and turbulent velocity profiles with intermittency as the weighting factor. With the symbols same as in Dey's presentation, the composite expression for the velocity profile is $u = (1 - \gamma)u_L + \gamma u_T$, where u_L is given by the Blasius solution, and u_T is given by the log-law. Predictions made thus agreed very well with experimental measurements.

M. Nandanan, of Aeronautical Development Authority, Bangalore, spoke on 'Role of transition prediction in advanced transport aircraft design'. The paper was very significant in that it was a status report on where the Indian aeronautical industry stood, in relation to design and development of advanced transport aircraft. The available design tools were discussed, and so were the areas that needed urgent indigenous development, transition prediction being one of them. Nandanan pointed out that 65% of the total drag in a typical transport aircraft is due to friction; therefore, any future design should aim at reducing this. This is only possible if the designer can succeed in keeping the flow over the wing laminar to as large an extent as possible. Nandanan then went on to describe the design methodology of wing aerofoil sections, for keeping the flow laminar to as large an extent as possible. A typical laminar flow airfoil designed by Aeronautical Development Authority was tested in the 1 foot NAL transonic wind tunnel to study whether the airfoil sustains laminar flow. The test data and the computational results showed good agreement, and the lift/drag (L/D) ratio was found to improve by about 41% when the transition strip was removed for the free transition case. The NAL wind tunnel does not have the facility of measuring transition location. Therefore, the transition predictor of Govindarajan and Narasimha was used to estimate the location of transition. This predicted that transition was likely to occur at 34% of chord, both on upper as well as lower surfaces of the airfoil.

Whilst the above was no doubt an impressive first effort, Nandanan also pointed out the immediate needs for further design work in the aeronautical industry. In so far as 2D design tools are concerned, the Indian aeronautical industry has capabilities for airfoil design and analysis. It also has capability of transition prediction. However transition predictors based on stability calculations, for the compressible flow case, are still not available. For the 3D case, codes are available for full potential flow calculations, and for (integral methods based) boundary layer analysis. The shortfalls are: first, it is necessary to be able to calculate the details of the (three-dimensional compressible) flow within the boundary layer; second, to be able to do 2D and 3D stability calculations for the compressible flow case; and third, to be able to make predictions for the line of transition, along the wingspan, based on the stability calculations. Nandanan also mentioned that cross-flow instability could be playing an important role in the transition of the flow past the designed wingspan; and, study of this kind of instability also needed to be brought within the purview of transition predictors that need to be developed in future.

R. Narasimha, of the Indian Institute of Science, Bangalore, spoke on 'Subtransitions in the transition zone'. He presented experimental evidence for the view that the transition zone may, under certain conditions, be considered to contain what he called sub-transitions. Such sub-transitions could occur in a variety of situations. For example, a boundary layer subjected to pressure gradient over part of the transition zone could show a kink in the intermittency distribution – or more precisely in a function of the intermittency that is a proxy for the spot width. Favourable pressure gradients in the beginning of the transition zone were more effective in producing such sub-transitions than those that occur later, presumably because the same gradient in a flow that is closer to the fully turbulent state can be expected to have a weaker effect than on a nearly laminar flow. Narasimha showed detailed measurements in some flows where the location of the subtransition as inferred from intermittency coincided with abrupt changes in local boundary layer parameters. Inference of sub-transition in some flows reported by Blair, made on the basis of an analysis of indirect experimental evidence, was later confirmed by direct measurements of intermittency. Narasimha attributed such sub-transitions to rapid changes in spot parameters, which could occur in a flow if it went from sub-critical to supper-critical and vice versa depending on the pressure gradient it is subjected to, especially in the early part of the transition zone. Changes that occur on three-dimensional surfaces could also be instances of sub-transition. For example, on an axisymmetric body, a spot would first propagate as on a flat plate, but would eventually wrap around the body and propagate as a sleeve, as pointed out by Rao. This leads to a marked and relatively abrupt change in the nature of the intermittency distribution, which can now be predicted by a consistent theory, and could also be considered a kind of sub-transition. Narasimha emphasized that as interest grows in the transition zone in more complex flows, greater care would have to be given to the occurrence of such sub-transitions.

P. K. Sen spoke on his work with Srinivas V. Veeravalli on 'Stability of an organized disturbance in a turbulent boundary layer'. Here, the use of hydrodynamic stability theory in understanding the dynamics of wall bounded turbulent flows was discussed. This approach has remained somewhat dormant since the works of Reynolds and Tiederman, and Reynolds and Hussain. Reynolds and Hussain derived an extended Orr–Sommerfeld equation to describe the evolution of an organized disturbance in turbulent flow, based on what they called the Newtonian eddy model. This equation was independently derived in the present work by an alternative more compact route, using an isotropic eddy viscosity model. Although one obtains near neutral modes with this equation, there are no unstable modes. A further extension was made in the present work to incorporate a more general anisotropic eddy viscosity model. The final disturbance equation thus obtained does yield an unstable wall mode, and, the results seem to mimic some key features of fully developed wall turbulence. Numerical results were reported for the flat-plate turbulent boundary layer. The eigenfunction, the root mean square distributions of the organized disturbance velocities $\tilde{u}$ and $\tilde{v}$, and, the $\overline{\tilde{u}\tilde{v}}$ profile, are similar in form to those obtained for the case of the laminar boundary layer. So also is the value of the phase speed which is $c_r \simeq 0.3$. Thus both phenomena appear to be governed by the high shear region near the wall. The production peak, corresponding to the organized disturbance problem, occurs at $y^+ = 12.5$ which agrees well with the location reported in past experiments on the turbulent boundary layer. The band of unstable wave numbers also matches well with the turbulent u' power spectra measured (as part of the present work) in the buffer and log regions of a flat-plate turbulent boundary layer.

I. S. Shivakumara, of UGC-DSA Centre in Fluid Mechanics, Bangalore University, spoke on his work with N. Rudraiah on 'Effect of throughflow on Rayleigh–Benard convection'. Onset of convection in a horizontal fluid layer with throughflow in the vertical direction was investigated for different types of hydrodynamic boundary conditions (i.e. rigid–rigid, free–free and rigid–free boundaries). The critical Rayleigh numbers were obtained numerically using the Galerkin technique. It was found that throughflow, irrespective of its direction, makes the system more stable when the boundaries are of the symmetric type. However, when the boundaries are of different types, a small amount of throughflow in one particular direction destabilizes the system depending on the value of the Prandtl number P_r. It was further noted that the destabilization is small for $P_r \approx 1$, and increase in the same causes decrease in the critical Rayleigh number. Also, it was found that increase in magnitude of throughflow increases the critical wave number.

M. Gaster gave the last talk on 'Prospects for active control'. Here, some possible schemes for wave cancellation based on active control were discussed and reviewed. A single monochromatic wave, introduced in a controlled manner, could be cancelled further downstream by introducing another disturbance source that is made to be in opposite phase by active control. However, although this is an interesting laboratory experiment to perform, this does not automatically guarantee transition control; because, in a real life situation the intrinsic disturbances leading to transition expectedly have a much more complex composition as compared to a single monochromatic disturbance wave as in the case of the typical laboratory experiment. Active cancellation of an arbitrary disturbance is still a far cry. Nevertheless, Gaster showed some analytical and experimental results which are very promising. These pertain to generation of a wave packet and active cancellation of the central part of the wave packet by using a piston plunger arrangement downstream. The approach seemed very promising and is a major contribution towards achieving the goal of active control.

After Gaster's talk, R. Narasimha gave an overall summary of the entire conference.

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M. S. Swaminathan Research Foundation

Womens' Biotechnology Park

Under the sponsorship of the Department of Biotechnology, Government of India, the scope for establishing a Womens' Biotechnology Park is being studied. The aim of the Park is to provide women professionals opportunities for taking to a career of self-employment in the field of biotechnology. The choice of enterprises will be made on a careful assessment of their ecological, economic and social sustainability and desirability. The entrepreneurs will be enabled to undertake the manufacture and marketing of various biotechnological products and services with the help of institutional credit. The management of the Park will be in the hands of the stakeholders. In its structure, the Park will adopt the principle of decentralized production supported by key centralized services. A minimum of Bachelor's degree will be needed to master the production techniques.

Interested women entrepreneurs may send their names and postal address to Dr (Ms) Sudha Nair, Convenor, Womens' Biotechnology Park, M. S. Swaminathan Research Foundation, 3rd Cross Street, Taramani Institutional Area, Chennai 600 113, by 21 May 1997. Those interested will be invited to a stakeholder's meeting early in June 1997 where the details of the proposed Biotechnology Park will be presented for discussion and finalization.

IPR and microorganisms

Suman Sahai

Gene Campaign, the group which has worked actively on the issues of Intellectual Property Rights on biological materials, held a National Seminar on Microorganisms on 13 February 1997 in New Delhi.

The objective of the seminar was to define what kind of IPR position on microorganisms would be in India's interest in the context of the WTO/TRIPs. A need for such a national level discussion became necessary since it was widely felt that the government had accepted patents on microorganisms without either a debate on the issue or any real understanding of the issues and their implications.

The objective of the seminar was also to explore how India could re-define its position within the WTO so as to better protect its national interest.

The seminar chaired by S. K. Sinha, National Professor and former director of the Indian Agricultural Research Institute, was attended by a wide cross section of people. These included representatives of the ministries of Agriculture, Commerce, Industry, Defence and Science & Technology; APEDA; representatives of private and public sector industry; scientists from national institutions like CCMB, Hyderabad; NBRI, Lucknow; IMTECH, Chandigarh; TERI, Delhi; IARI, CSIR and from universities and research institutions. In addition, there were participants from the voluntary sector, trade unions, the Biotechnology Association, patent attorneys, environment officials from some African nations and students.

The participants felt that the issue of microorganisms was little understood. It was apparent that not enough thinking had gone into what India's position should be on microorganism patents. The subject has been discussed for the first time at this meeting and it was felt that many more discussions were needed at various levels before a policy decision could be taken. There was a strong view that India should not act in haste and accept things on which there was so little understanding.

The following are the major recommendations that emerged from the meeting.

The country must immediately establish ownership over its genetic resources in accordance with the Convention on Biological Diversity. This was urgently needed to stop its plunder by foreign agents which were smuggling out bioresources in the legal limbo that exists in the absence of national legislation. The nodal ministry, the Ministry of Environment was urged to draft a law without losing any more time. Other nations like the Philippines, Indonesia, Australia and Ecuador have already got laws establishing ownership.

National policies to protect the national interest in matters relating to biodiversity and our wealth of national flora must be clearly defined. Our policies should be formulated to protect this national interest. We should retain enough flexibility in our approach to review and change our policies from time to time in response to changes in the national and international situation.

Economic considerations must override scientific and technical arguments in deciding a patent policy for microorganisms. This was essential since the demand for patents has arisen in a trade forum like WTO/TRIPs and is to be seen in the context of defending economic interests primarily. Therefore the economic, not the scientific framework must be used in deciding policy.

There are inbuilt contradictions between the concerns of developing and developed countries. For the former it is food security, for the latter it is commercial interest, hence they cannot have the same patent regimes.

It is imperative that we give priority to social considerations and the public good over the commercial interests of corporations that are the real beneficiaries of patents.

A national level Bioresource Board should be set up to govern and regulate bioresource use and policy. This should consist of scientific and technical experts, representatives of local communities, members of government departments, NGOs, experts in law and foreign trade and members of the media.

Importance of microorganisms

Given the vital importance of biotechnology to the future of the Indian economy, any policy decisions on biological resources, specially Intellectual Property Rights, must be very carefully thought through.

Microorganisms which play an important role in biotechnology are crucial to modern agriculture, and industry. Given their role in biomining, in resource recycling, bioremediation, in the production of alternate sources of fuel, such as biogas, ethanol and solar energy and in enzyme technology, their unrestricted use is vital to ensure the high level production base needed for national development. Any possible patent regime must be discussed in depth before we take a hasty, uninformed decision.

Current status

Indian institutions and scientists are poorly prepared to function in a microbe patent regime. There is widespread patent illiteracy at all levels in the country. There is a high level of ignorance about drafting and filing for patents among scientists. CSIR and ICAR are not providing any technical assistance. It is, therefore, of vital importance that scientists, attorneys, judges, government functionaries and patent office staff are trained in technical matters relating to patents if we are to meet the challenge posed by the new regime.

Since it is not very clearly understood as to how the new patent regime will affect our agriculture and industry, many more discussions and brain-storming sessions must be held before we take a stand on the matter. Much greater technical understanding is needed, for even simple things like what constitutes a patentable organism.

Action needed

There is an urgent need to document and characterize our micro flora. India is rich in microbes. Not even 1% has been identified so far. We need to characterize our microorganisms to lay claim to our resources and know how to use them.

The government has to invest in this area to boost the fields of biosystematics and taxonomy.

There has to be better coordination between various government departments and universities which are involved in this field, so that technical skills can be maximized effectively.

There is an urgent need to create our own depository for microorganisms. Presently, India does not have such institutions as the American Type Culture Collection (ATCC), and our valuable microbial germplasm continues to be deposited in foreign countries. In addition, we have to develop our own skills for characterization, otherwise our samples will continue to be sent outside for identification and characterization.

Experts suggested that India can reject patents on microorganisms without in any way jeopardizing its position in the WTO. There is sufficient scope within the WTO framework for nations to exercise flexibility in defining patentable subject matter. They can for example, exclude from patentability those categories which would offend public order or morality. The 'Odre Public' and morality clauses have been successfully used in Europe to oppose patents already granted by the Europe Patent Office. The possibility of invoking this clause to protect our indigenous plants and knowledge must be fully explored. Even today, there is no patent harmonization in western countries, mainly on account of objections from society on various issues, particularly morality.

It was widely felt that India should not accept patents on microorganisms at this stage. Patents favour only the patent-holder, not the common man, not the scientific community, not the larger community. Patents also block the flow of information and materials. Scientists do not have free access to microorganisms for research purposes. Studies have shown that strong patent regimes slow down the discovery of new drugs. It is wrong to say that patents are the only way of rewarding the scientific community or of providing them with a much-needed infusion of funds for research. In fact, over 80% of patents are held by corporations, not by scientists. Moreover, patents on microbes will lead to patents on plants and animals and all life forms, which is unacceptable.

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Sacred groves of India – Vanishing greenery

G. Harikrishnan Nair, K. Gopikumar, Pramod G. Krishnan and K. K. Sunil Kumar

Love and respect towards nature has been an ancient Indian tradition. Affection towards nature was as zoolatry (worshipping of animals), thottam (considering plants and animals sacred), etc., which in turn led to a sort of prudent conservation. Religious beliefs, traditions and customs of Indians bear an allegiance in restricting the exhaustive use of natural resources. An understanding of the conditions under which human societies evolved, effective methods of prudent use of resources and of the circumstances under which these practices broke down is considered very important in our endeavour to steer ourselves in to a course of sustainable utilization of earth's resources[1]. There are several examples of such social restraints on resource utilization. In Bhandura district of Maharashtra, the traditional fishing had never disturbed the spawning aggregations of freshwater fishes in the hill streams. Another example for the traditional system of refugia for natural resources is the network of sacred groves seen throughout India[2].

Sacred groves are patches of vegetation preserved on religious grounds. The area of a sacred grove varies from a few trees to some acres. Each grove has a patron deity and folklore associated with it. Any sort of damage to these vegetational sanctities was considered a sin by villagers or the people of that locality.

Distribution

There are only four regions important for groves in India[3]. These include the Khasia and Jaintia hills, the Western Ghats of South India, Aravalli hills and Sarguja, Chanda and Bastar areas of central India. In India the maximum number of sacred groves are seen in the states of Kerala, Maharashtra, Madhya Pradesh, etc.

Importance

Sacred groves are ecologically and genetically very important. They are the abodes of rare, endemic and endangered species of flora and fauna. Besides, they serve the function of preserving the genetic diversity of even the common species of trees. Ancient teak (*Tectona grandis*) forests are reported from two sacred groves of Maharashtra, though teak has disappeared from their vicinity. These teak specimens exhibit genetic variation and therefore proved to be of great value in the future tree breeding programmes[4]. Wild cultivars having better pest resistance and productivity are also reported from sacred groves. They may also become useful in future in the genetic improvement programmes[5].

In Allepey district of Kerala, natural forests are encountered only in sacred groves that remind us of a great past. Certain new plant species have been identified from some sacred groves. Presence of a new woody climber (*Kunstleria keralensis*) had been reported from a sacred grove in Kerala[6].

The value of sacred groves is immense. They are good sources of a variety of non-wood products like fruits, fodder, fuel wood, fatty oils, spices like pepper, cinnamon and nutmeg, medicinal plants, etc. A detailed account of the medicinal plants, wild cultivars and endemic species found in a sacred grove (Iringole kavu) of Kerala state has been reported[7].

The faunal wealth of the sacred groves is also worth mentioning. An ornithological survey in a sacred grove of Kerala (Mukkuthala kavu, Malappuram) could identify seventy species of birds[8]. However, detailed faunal studies are still lacking from sacred groves. Besides these, the amenity value, roles of sacred groves in water conservation and their effect on microclimate of the region, etc. are also important and deserve special attention in future studies.

Present scenario

Our ancestors were fully aware that the natural resources which sustained them must be conserved for the sustenance of future generations. But the inconsiderate and self-centered modern man is exploiting the natural resources without giving heed to the well-being of younger generations. Sacred groves are the victims of this grim tragedy. During the early period, collecting even a fallen branch from a grove was taboo. However, the scenario has changed now. The increasing human population, along with the deteriorating religious faith, had led to the relentless exploitation of these vegetational sanctities.

Out of the four sacred groves in the Panshet reservoir catchment of Maharashtra, Central India, one at Shirkoli and another at Gondelkhal, both of fifteen hectares each, were felled in 1956. The other two sacred groves, one at Tav and the other at Mangaon were also reported to be in danger[4]. In Maharashtra, inam groves (groves in which no deity resides) are said to be destroyed to a large extent.

In Kerala also, human interference has caused severe damage to sacred groves. The study on the ecological aspects of Mukkuthala kavu (Malappuram Dist.) revealed that this grove has rich floral wealth (about eighty-nine plant species were identified of which forty-three are arborescent). They include rare, endemic and endangered species[9]. Among the species identified, there were fifty medicinal plants and six wild relatives of cultivars. Unfortunately this biological richness is being eroded away by various biotic interferences. Based on the extent of disturbance, two zones, viz. disturbed and undisturbed, were clearly identified in this area. Phyto-sociological studies and soil analysis carried out separately in these two zones throw light on the plight of this grove. In the disturbed zone, the soil nutrient status and vegetational cover is being depleted day by day due to biotic interferences in the form of grazing, fuelwood collection, etc. Recently the one and only specimen of *Syzygium travancoricum* of this grove, which is a rare species was felled by the temple authorities. This indicates the irreverent attitude and lack of awareness among the people regarding the importance of sacred groves.

Ecophysiological studies conducted in Mannampurath kavu (Kasargod Dist.) also emphasize the importance of complete protection and the need of public awareness for the existence of such relict communities[10]. This grove is also bountiful in its floral aspects with 61 species of angiosperms. The study revealed that the species composition and density shows marked difference with the degree of disturbance. The lion's share of this grove is in the disturbed zone. The soil nutrient status and vegetational cover is dwindling rapidly and in no time this area will become barren. These studies point to the fact that sacred groves are no longer going to have the privilege they had in the past.

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Gene silencing: A problem in transgenic research

Tapan Kumar Mondal, Pradip Kumar Kundu and P. S. Ahuja

Transferring a gene from one plant to another is an area of immense importance in transgenic research today. Successful gene transfer depends on the integration and expression of the foreign DNA within the plant cell. Some recent reports suggest that if the foreign DNA, which is homologous to the endogenous gene gets integrated into a plant chromosome, then both the transgene and the endogenous gene are likely to become inactivated[1]. Even if multiple copies of the transgene are integrated, all or some of the copies become inactive. Such a phenomenon of gene inactivation in response to transgene transfer is known as gene silencing or transgene inactivation. Genes can be inactivated/silenced due to sequence homology either between the transgene and the endogenous gene or among transgenes themselves. The sequence homology may be present either in the promoter region or in the protein coding region. This fact is broadly defined as the 'homology dependent gene silencing'. Depending upon the mode of gene silencing, the mechanism can be classified under three types namely: (i) *cis*-inactivation, where silencing occurs among multiple copies of the transgene integrated either as inverted or as direct repeat on the same chromosome; (ii) *trans*-inactivation, where silencing occurs either in the transgene or in the endogenous gene when the integration occurs on a different chromosome. It can be either at allelic or non-allelic chromosomal locations with respect to the transgene insert; (iii) co-suppression/sense suppression, when expression of both the endogenous and the transgene coordinately gets suppressed. A well-documented example was reported with chalcone synthase (CHS) gene which is responsible for flower colour in petunia plant. When this gene was transferred in petunia to get a darker flower, interestingly, some white flowers were produced. This was attributed to the fact that the endogenous and the transferred CHS gene were silenced in white flowers[2].

Inactivation of a gene might occur at the transcriptional or at post-transcriptional level and different models/hypotheses were put forth for its explanation. Transcriptional gene inactivation can be explained by ectopic pairing[3], where the pairing of chromosomes occurs between an allelic and non-allelic (ectopic) homologous sequences due to some 'homology searching mechanism' present in the plant system. The ectopic pairing, in contrast to the normal crossing-over, is transient and does not involve any exchange of genetic material but occurs between very small homologous segments of non-homologous chromosomes. Rather, there is an exchange of some chromatin material causing heterochromatization which is known as a non-functional segment of chromosome for gene expression. It is also believed that some methylation does occur during ectopic pairing.

Transcriptional gene inactivation can also be explained by paramutation, in which the gene expression of one allele can be changed by exposure to another allele in a way that persists even after segregation of the two alleles. The allele that induces mutation to another allele (paramutable allele) is termed as paramutagenic allele. Paramutation was first observed in R locus of maize and was found only in plants[6]. Thus, the transgene or the endogenous gene or multiple copies of the transgene become paramutagenic inducing mutation in the corresponding homologous allele present in the plant genome. Paramutation also involves hypermethylation at the promoter region of the homologous DNA segment and causes gene suppression.

Post-transcriptional level was explained by (i) biochemical switch model[4], where accumulation of high level of gene product, i.e. RNA, reaching the critical threshold level, due to presence of more copies of the same gene leads to RNA degradation by RNAase. The degradation induces a type of feedback mechanism causing methylation of the homologous DNA sequences which causes suppression of the respective DNA. (ii) Antisense RNA hypothesis[5]. According to this hypothesis, sometimes antisense RNAs may be produced inside the plant cell, when the endogenous plant promoter is located at 3′ end to the transgene insert or they may be generated from the unintended RNA (abberant/faulty RNAs) produced by a plant-based RNA dependent RNA polymerase. These abberant RNAs are produced as a result of (a) formation of secondary structures of DNA during transcription, (b) methylation of the corresponding DNA (sense strand) and (c) delay in RNA processing when heterogeneous nuclear RNA of transgene and its endogenous gene gets accumulated due to the same processing track. Once the antisense RNA is produced, it pairs with the corresponding sense RNA and blocks the translation. Further, this double stranded RNA structure is degraded by a double strand specific RNAase, present inside the plant cell.

It is noteworthy to mention that at a time, more than one mechanism may operate simultaneously to silence a gene. Methylation plays a vital role in gene silencing both at transcriptional and post-transcriptional levels. The methylation occurs at 5′ position of cytosine residue by an enzyme called DNA methylase. In eukaryotes over 90% methylation occurs at CG dinucleotide sequences. But how does the *de novo* methylation occur? Two different nucleic acid interactions are believed to cause *de novo* methylation: DNA–DNA or DNA–RNA pairing (Figure 1).

The DNA–DNA pairing forms a hairpin structure which triggers the DNA methylase to cause methylation at both the strands. The DNA–DNA pairing and methylation was first observed in a filamentous fungus *Ascobolus immersus*, where the duplicated DNA sequences pair among themselves and form a hairpin structure leading to transcriptional inactivation of the gene and the process is called 'methyl induced premeotically' (MIP). DNA–RNA pairing also serves as a signal for *de novo* methylation. It has been observed in the transgenic plants which contain viral cDNA that after the production of RNA, from viral cDNA, the DNA becomes

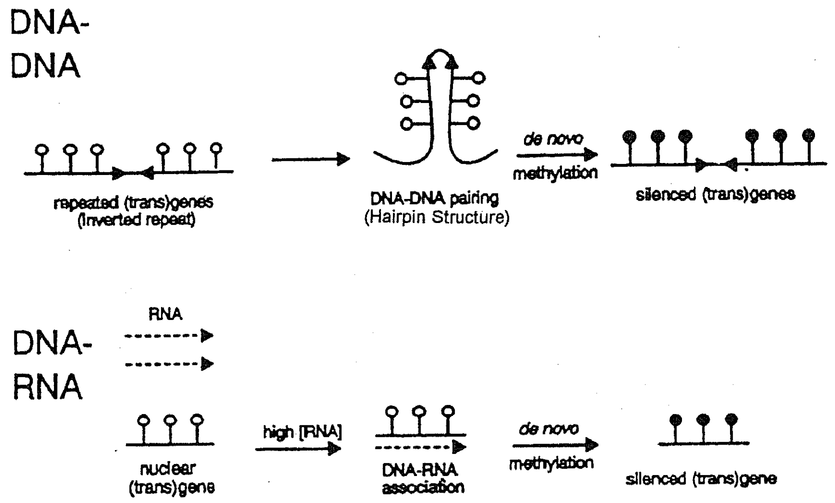

Figure 1. Hypothetical models of nucleic acid interaction involved in transgene silencing (Reproduced from ref. 7 with permission).

methylated probably through DNA–RNA pairing. The above observations were extended further to plant gene suppression involving DNA–RNA pairing which possibly triggers *de novo* methylation.

How do plants recognize the transgene as a foreign element and why do they have a tendency to suppress the homologous gene? It is well known that plants recognize an invasive DNA as 'foreign element' because of different CG contents[7] and like other living organisms, the plant cell also tries to eliminate the foreign body by activating its defense mechanism, which involves DNA methylation. DNA methylation serves as a useful tool in plants against the viral multiplication and also to prevent expression and spread of some transposable elements.

How can one overcome the transgene silencing? Gene silencing can be checked successfully firstly by using a drug named 5-azacytidine which is a modified cytosine base, incorporated during DNA replication. This drug is added in tissue culture medium during regeneration of the transgenic plant. Secondly, single copy gene insert can be selected among the transgenic progenies. However, further research is required to combat with this undesirable problem encountered during transgenic plant research.

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The India–Asia collision warps and thaws Tibet's bowels

A. V. Sankaran

The Himalayas, along with the Tibetan Plateau, formed as a result of a classic continent–continent collision that took place about 50 million years (Ma) ago between India and Asia[1], is an uniquely elevated region of the Earth. The colliding boundaries of the two continents, forms today, the well-known Zangbo Suture Zone which coincides with the Yarlung–Zangbo river valley. The complex geological structure of the region has given rise to considerable debate among the earth scientists trying to interpret its orogenic evolution. According to some of them, the Indian plate, along with the crust and mantle below, is sliding underneath the Asian Plate, while a few others believe that the two plates are colliding head-on; and these tectonic processes are still active. Whatever be the mode of plate movements here, they have warped the colliding faces and pushed the crust deep down and created features, quite unique for the lithosphere of this plateau. For example, the region has an anomalously thick crust (almost twice the normal continental thickness), and an unusually high amount of heat flow. Earlier geophysical surveys have indicated that the unusual crustal thickness here is due to convergence during the collision of the two continents[2], while the abnormal heat flow has been attributed to the existence of molten granite at a depth of 10 to 20 km[3].

During the last couple of decades, this warped and contorted region had attracted a few international teams of geoscientists who undertook surveys to evaluate its geology and tectonic evolution. The latest to carry out such joint studies, is a team of scientists from USA, Canada, Germany and China. They undertook detailed geological and geophysical investigations to bring out an indepth profiling of Tibet and the Himalayas and advance existing

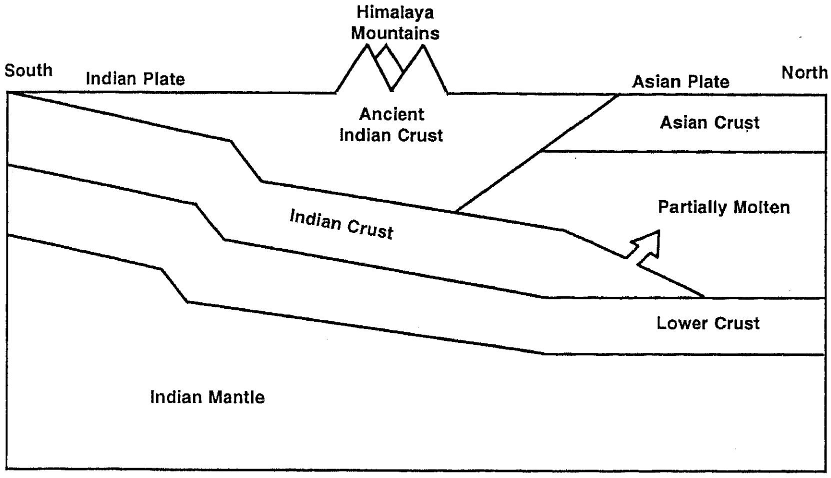

Figure 1. The India–Asia collision showing underthrusting and melting of the Indian crust and partial melt-zone under Tibet whose crust is twice the normal continental thickness.

knowledge of the orogenic evolution of the region. Called the 'INDEPTH' Project, this team of investigators spent the summers of 1992, 1994 and 1995 carrying out their surveys along the well-known Yadong-Gulu rift, the largest of several north-south rifts extending across the Himalayas and southern Tibet. They did seismic profiling using reflected waves from explosions set off in boreholes and also studied waves from distant earthquakes to calculate the density and temperature of the interior zones; in addition, they also conducted measurements of magnetic and electric fields to understand the electric properties of the rocks below, apart from carrying out routine surface geological survey.

The data gathered by the INDEPTH team[4–8] have further confirmed many of the observations by the earlier workers[2,9–14]. The combined studies by various teams over the years, have brought out some of the geological and tectonic aspects peculiar to this region. The highlights of their findings are (Figure 1): (i) The India–Asia collision has been in progress for about 40–50 m.y; (ii) A partially molten midcrustal material exists below 15 km depth which extends through a greater part of Tibet and petering out south of the Zangbo suture zone. (iii) The collision of these two continental plates resulted in the melting of the Indian and Asian crusts, obliterating their identity beneath Tibet. (iv) The Indian lithosphere extends as a mechanically continuous unit northwards, along with the upper mantle from northern India to the centre of Tibetan plateau. (v) In this process of underthrusting of Indian lithosphere into southern Tibet, its attached crust 'warms and partially melts, thereby contributing to a partially molten midcrustal layer[4]'. This melt zone is widely developed beneath the northern Yadong–Gulu rift and possibly much of southern Tibet[5,7], at depths of 15–18 km; however, the thickness and spread of the melt is not clearly demarcated[5] by the teams. (vi) Partial melting of the Tibetan crust induced by the crustal thickening is the most likely source of magma, which in composition may be granitic along with water[5,6]. (vii) The well-known Moho discontinuity, which demarcates the transition from the heterogeneous crust to the homogeneous mantle, is noticed along the entire length of the area surveyed at 70 to 80 km which is deeper than the customary depths for the continents. Existence of Moho at such uniform depth below Tibet implies that the crust/mantle boundary here is essentially flat[4]. (viii) The undulatory, discontinuous nature of the rift system studied (Yadong–Gulu rift) suggests that the magmas have invaded along the pre-existing structure or that the post-intrusion deformation continued for a long time. (ix) The high electrical conductivity of the middle crust in this region indicates that there is a regionally interconnected fluid phase in the crust[8]. (x) The regionally developed, weak midcrustal partial melt layer also explains why the Tibetan plateau is essentially flat.

The findings of the INDEPTH Project are indeed good breakthroughs in Tibetan Himalayan geology and one can hope that they will stimulate some revisions of the views about collisional orogens in other parts of the world; however, a serious lacuna about INDEPTH's present data relate to the fact that their studies covered only a small part of Tibet; the investigators appear to be aware of this as they are planning to extend their surveys over a wider area in the coming years.

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Caprine arthritis encephalitis in Indian goats

Caprine arthritis encephalitis (CAE) is a viral disease of goats, characterized by encephalitis in kids and arthritis in adult goats. It is caused by caprine arthritis encephalitis virus (CAEV), a retrovirus of subfamily Lentivirinae[1]. Other members of this subfamily are – maedivisna virus (MVV) of sheep, equine infectious anaemia virus (EIAV) and human immunodeficiency virus (HIV). CAE has not so far been reported from this country.

1134 goat sera were screened to detect antibodies against CAEV antigen by agar gel immuno-diffusion and enzyme-linked immunosorbent tests. About 18% serum samples were found to be positive, indicating a prevalence rate of 0.179 for CAE in the target population. Thirteen cases of nonsuppurative arthritis, characterized by enlargement of carpal joints containing 15–50 ml clear to turbid yellow synovial fluid with high cell counts (15,000–1,00,000 cell/ml) predominantly of mononuclear cells were also recorded on clinical, gross and histological examination (Figure 1). Cytoplasmic antigen of CAEV could be demonstrated in the cells of synovial fluid by indirect immunofluorescent and immunoperoxidase tests. The arthritis was classified into exudative, proliferative and degenerative types. Proliferation of synovial membrane forming villus projections, massive infiltration of mononuclear cells and development of germinal centres around blood vessels and in the parenchyma of the tissues; necrosis of the joint structures with fibrosis and calcification were the salient histological features.

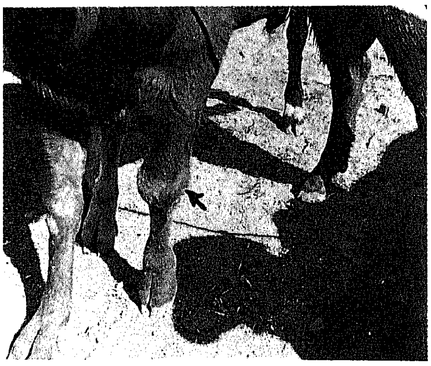

Figure 1. Lentiviral arthritis: enlargement of left carpal joint.

The CAEV was isolated from synovial membranes of 2 seropositive goats. The new isolate CAEV_{bp} (named after Bhopal) exhibited genetic and phenotypic similarities with CAEV_{co} (American isolate). The isolate induced multinucleated giant cell formation in goat synovial membrane (GSM) monolayers, unable to replicate in sheep choroid plexus cells, and its amplification of 512 base pairs from *gag* sequences on PCR identified the new isolate as a virus of caprine origin. These findings are in conformity with those of previous workers[2,3]. The virus-specific protein p25 and gp135 were also precipitated in macrophage and GSM cultures by immunoprecipitation. Ultrastructurally, different stages of virion formation and maturation were observed in GSM cells. These observations confirm the existence of CAE for the first time in Indian goats.

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In vitro flowering and back crossing of *Brassica* intergeneric hybrids

Intergeneric hybridization between the related wild species and cultivated brassicas offers vast scope for the improvement of crop brassicas, especially in providing cytoplasmic male sterile (CMS) lines. When hybrids are obtained in culture by rescuing embryos and transplanting in soil is difficult, it would be advantageous to induce flowering *in vitro* because of the following reasons – i) Meiotic analysis is possible to determine homology of different species/genera; ii) Inheritance of floral/fruit attributes can be determined and iii) Backcrossing of hybrids can be attempted *in vitro* to obtain backcross progenies quickly if pollen of male parent is always available. It has recently been suggested to advance segregating population by selfing *in vitro*[1]. Flower initiation and development is known to be promoted by environmental conditions such as pH, day length and different growth regulators[2]. Among the interspecific or intergeneric hybrids of *Brassica*, *in vitro* flowering has been obtained only in hybrids between *Enarthrocarpus lyratus* and crop brassicas[3]. There are no reports of *in vitro* backcrossing in wide hybrids of crop plants. Here we report *in vitro* flowering of two intergeneric *Brassica* hybrids (*Erucastrum abyssinicum* × *Brassica*

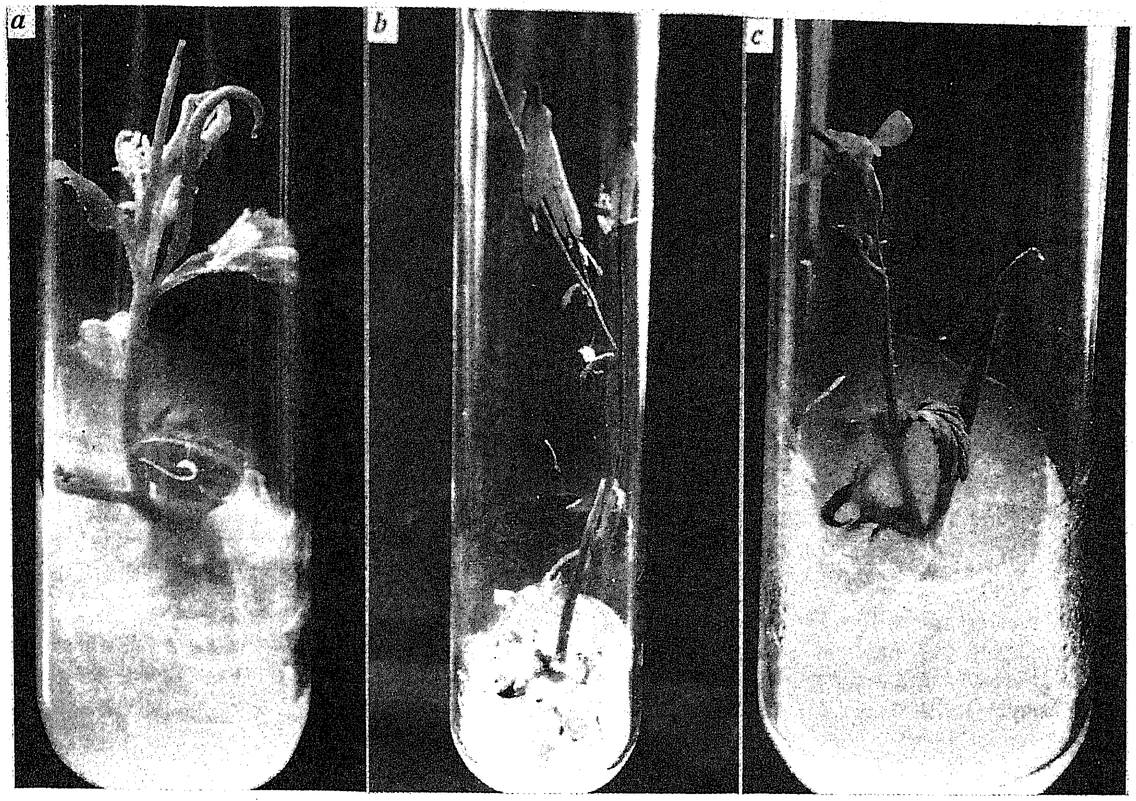

Figure 1. *a*, Hybrid EO1 plant in culture with flowers and siliquae; *b*, Hybrid EJ1 plant with well-developed siliquae. Note the abscised petals; *c*, Hybrid EJ2 plant with flower, exposed pistils and roots.

Table 1. Results of backcrossing of hybrid EJ1 and EJ2 with *B. juncea in vitro*

Hybrid	No. of pollinations	No. of BC_1 seeds cultured	No. of BC_1 seeds germinated	% Seed germination
EJ1	23	39	–	0.0
EJ2	59	28	2	7.14

oleracea and *E. abyssinicum* × *B. juncea*) obtained through sequential culture of ovaries and ovules. *E. abyssinicum* × *B. juncea* hybrids were backcrossed *in vitro* and BC_1 seeds were obtained.

Ovaries at 10 days after pollination were excised and washed thoroughly in running tap water. They were surface sterilized in 0.1% aqueous mercuric chloride solution for 2–3 minutes and rinsed 3–4 times with sterile distilled water. Surface sterilized ovaries were cultured in MS[4] medium + 400 mg/l casein hydrolysate. Only one ovary was placed in one culture tube. In all, 96 ovaries were cultured in the cross *E. abyssinicum* × *B. oleracea* and 121 in *E. abyssinicum* × *B. juncea*. The cultures were kept under fluorescent light (3000–4000 lux) at 25 ± 2°C. An alternating 16 h light and 8 h dark period was maintained. After 15–20 days in culture when ovaries turned brown, ovules were dissected out from the ovaries and turgid ovules were cultured in MS medium + 5% sucrose + 1 mg/l kinetin + 400 mg/l casein hydrolysate. The number of turgid ovules per ovary ranged from 1 to 13 in the first cross and 0 to 4 in the second cross. Ovules from one ovary were placed in one tube and kept in dark till the embryos grew out. Hybrids obtained by sequential culture of ovaries and ovules were maintained *in vitro* by nodal segment culture. Nodal segments were cultured in MS medium + 0.5 mg/l BAP and kept in continuous light (3000–4000 lux) at 25 ± 2°C. Shoots of the hybrids were rooted in MS medium + 0.1 mg/l NAA.

Using the flower buds produced *in vitro*, cytology of the hybrids could be done. Flower buds were fixed in Carnoys solution (alcohol:chloroform: acetic acid::6:3:1) and anthers squashed in 2% acetocarmine. Pollen fertility was studied using acetocarmine staining.

E. abyssinicum × *B. juncea* hybrids were backcrossed *in vitro* to *B. juncea*. *B. juncea* buds were collected one day before anthesis, surface sterilized with 0.1% $HgCl_2$ (w/v) and implanted in agar medium (MS). The tubes containing flower buds were kept in continuous light (3000–4000 lux). On anthesis the next day, the flowers with dehisced anthers were used to pollinate the stigmas of the hybrids under aseptic condition.

In the sequential ovary–ovule culture, 52.5% ovules germinated (hybrid seedlings) in the cross *E. abyssinicum* × *B. oleracea* and 8.3% in *E. abyssinicum* × *B. juncea*. The hybrids were obtained off season. They were therefore, maintained *in vitro* by culturing nodal segments in MS medium + 0.5 mg/l

BAP. New shoots regenerated both from axillary buds and callus developed at the base of the nodal segments cultured. New shoots were subcultured in the same medium after every 15–20 days.

In the cross *E. abyssinicum* × *B oleracea*, three hybrids (designated as EO1, EO2, EO3) and in the cross *E. abyssinicum* × *B. juncea* two hybrids (EJ1, EJ2) were maintained up to maturity. Hybrids flowered *in vitro* (Figure 1 *a*). Frequency of new shoots bearing the floral buds in EJ hybrids was 2.5% during the maintenance. Later siliqua also developed (Figure 1 *a*, *b*), but the siliqua did not contain any seed. It took about 45–60 days for the first appearance of floral buds from the time of ovule germination. Flower buds *in vitro* are initiated early with the addition of BAP under specific environment[5].

Shoots of the hybrids in both the crosses were rooted on MS medium supplemented with NAA (0.5 mg/l) (Figure 1 *c*). In EO hybrids (EO1), 25% of rooted shoots flowered and developed fruit. Frequency of rooted shoots bearing flower buds was 84.6% in EJ1 and 52.3% in EJ2. This indicated that not only cytokinin, but auxin can also induce flowering *in vitro*. It was observed that frequency of flower bud initiation was more in auxin (NAA) supplemented medium than in cytokinin (BAP) supplemented medium. This may be because of endogenous cytokinin level which is higher when roots are induced and this enhances the initiation of flower buds.

EO hybrids produced white flowers which is a characteristics of male parent *B. oleracea*. On the other hand, EJ hybrids produced yellow flowers and were of intermediate size. These floral characteristics of EO and EJ hybrids proved their true hybridity. Some of the floral buds in both EO and EJ hybrids did not open and were apetalous, others dried up before anthesis. Higher levels of cytokinin may lead to small sepals, petals and reduced pedicel length of the flowers[5]. The hormonal requirements at various stages of flower development differ and might be the cause for this kind of abnormal flower development[2]. The anthesis of the flower buds after their initiation in the hybrids can be increased by transferring the plants to a medium containing less auxin/cytokinin or a hormone-free medium. EO hybrids (EO1) had 25 chromosomes in the PMC, the commonest association of chromosomes being 1 IV + 7 II + 7 I. Pollen fertility was 6.5%. On the other hand, EJ hybrids (EJ1) had 34 chromosomes, the commonest association of chromosomes being 1 IV + 12 II + 6 I. Pollen fertility was 10.9%. Hybrids had the expected number of chromosomes in their PMCs which indicated their true hybridity.

The ovaries enlarged after backcrossing. Ovules were excised 7–10 days after pollination and cultured in MS medium + 5% sucrose + 400 mg/l casein hydrolysate + 1 mg/l kinetin. As BC_1 seeds were obtained in both the hybrids (EJ1 & EJ2) of *E. abyssinicum* × *B. juncea* cross (Table 1), ovular fertility of the hybrids was confirmed. Only 2 ovules germinated out of 28 BC_1 ovules cultured in the case of EJ2. In one, the cotyledonary leaves emerged but the hypocotyl did not elongate. This dried up later. In the other, hypocotyl gave rise to a callus. The callus was subcultured in MS medium + 0.5 mg/l BAP for regeneration to take place. One BC_1 plant regenerated after about 60 days of continuous subculturing. Different media and culture methods such as ovary/ovule culture need to be tested for the rescue of BC_1 embryos. Ovary culture proved useful in the cross *Diplotaxis erucoides* × *Brassica napus* to obtain BC_1 seeds[6].

It took about 70 days to obtain BC_1 seeds from hybrid seeds. If stored or fresh *B. juncea* pollen is available, at least 5 backcross generations can be grown in a year using this technique of *in vitro* backcrossing. In each backcross generation, selection can be made for male sterility. The desirable alloplasmic lines can then be directly transplanted to the soil and thus alloplasmic lines can be easily obtained in a short period of time.

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Non-indexed citedness

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Two recent fact-finding investigations demonstrate that eponymal and indirect-collective citedness are very frequent and long-standing phenomena in the journal literature of physics, at present not indexed by Citation Indexes. In consequence of non-indexed literature citedness, the Indexes are not suitable for the measurement of real literature citedness. The meaning and value of quantified citation data of the Citation Indexes must be reduced, especially in the cases of the (mis)use of the data for the purpose of evaluating scientists as scientists.

COMMUNICATING scientists as authors of journal articles, right from the start and out of various motives have made reference to the works of other scientists bearing relation to their own work. These reference acts form bibliographic connections which have been shown for decades by the Citation Indexes in ever-wider fields of current science.

For a scientist, the Indexes show which references were made in the observed and processed literature to a work by himself or another scientist working in the same field, thus giving information on the progress of the related literature.

However, the grandiose (originally) bibliographic instrument published by the Institute for Scientific Information (ISI) views these bibliographic connections from the angle of the cited author and the cited work and regards and names the reference act as a citation 'received' by the cited work. However, following the publication of the Indexes, in this approach primitive quantification and measurement appeared (because it had become possible): research to determine how many citations were 'obtained' by the communication of a given scientist, by the articles of a journal, by the research work of a scientific institute, and so on.

Naturally, citation-counting measurement was followed by numerical comparison, and this in turn was followed by quantitative evaluation. The fashionably modern and current research trend which has had a big influence on science administration, at first known as 'citation analysis' but now as 'scientometrics' or 'informetrics', essentially still deals with these aspects. It is not unusual now for its indices calculated for individuals – the 'impact factor' and the 'citation index' – to determine the career of a scientist[1].

The raw material of this research is the data of the Indexes, that is, the reference stock printed in the text of articles separated from the main body of the text, and indexed by the ISI in the processed part of the literature as 'citations'. The concept of indexed citations, however, is narrower than that of formal references, because the ISI indexes as 'citations' only the formal references of direct and itemized nature.

Although the ISI now indexes as citations not only the *literature* references (i.e., published works), but also *verbal* ones ('personal communications'), it does not take stock of and show the *total real* reference acts contained in the articles of the processed journals.

Some of the non-indexed but real reference acts cannot be taken into account, but the majority of them can. It is not possible to take into account those reference acts (even if of a 'formal' nature), the objects of which are not given in a concrete or itemized form; e.g. in a single issue of an elite science journal we find 'and prior publications' 'and later papers', 'and previous papers', 'and related papers', 'and earlier papers'[2]. The non-indexed but real reference acts which can be taken into account are of two kinds: eponymal references and indirect – and often collective – references. Eponymal references (the so-called eponyma, e.g. 'Pauli exclusion principle') are of non-formal nature, existing in the main text of an article. The indirect-collective references (e.g. '... and the references cited therein') can be of a formal nature, existing in the text of a formal (and indexed!) reference act, or of non-formal nature, existing in the article's main text (possibly in the context of a formal reference).

It can be stated that the stock shown by ISI represents only the indexed-formal references out of the total-real reference stock. The two stocks are generally not identical, and where this is the case, the indexed-formal reference stock is always the smaller. It is this stock, smaller than the total-real reference stock, which the Indexes show and which is the basis of all the results and conclusions of the citation studies.

Right from the start, Citation Indexes and 'citation analysis' have been the target of severe and many-sided but ineffectual criticism, recently reviewed and summarized by MacRoberts and MacRoberts[3]. The existence of non-indexed but real references as a defect does not fig-

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ure in this criticism, nor is it found in Sandison's famous paper 'thinking about citation analysis'[4], or in the more recent article of Liu[5] reviewing the citation studies, or in the most recent corresponding discussion about citation analysis in *Nature*[6–12]. Nevertheless, the existence of formally non-cited eponyma in scientific literature had already been raised in 1974 in a critical letter of the physicist Goudsmit[13] in the famous literature case of the so-called Ortega Hypothesis in *Science*, launched by Cole and Cole with their citation analysis study[14]. Despite this, the literature phenomenon of non-indexed citedness did not appear as a point of criticism in any of the numerous communications dealing later with this citation analysis case.

The silence of the related literature to date concerning this point has obviously been due to the fact that no investigation had been made either on the nature or the frequency of this literature phenomenon, and in the absence of factual research data, the bibliometric and other '-metric' significance of its existence were not known. No investigation was made either of the non-indexed eponymal or of the non-indexed indirect-collective citedness in the literature.

In the last few years, two investigations of these phenomena have been made to determine their nature. The first fact-finding investigation, directed at non-indexed eponymal citedness, was made in the material of two elite physics journals: the general *Physical Journal (PHJ)* and the special *Journal of the Optical Society of America (JOSA)*. The research covered two years (1939, 1969) which are representative of the last, mature period of Little Science and of early Big Science, and in the case of *JOSA* was extended longitudinally to the period from 1934 to 1974, in five-year steps. The processed volumes were: *PHJ* 55, 56, 177; *JOSA* 24, 29, 34, 39, 44, 49, 54, 59, 64; the number of processed articles: *PHJ* 922, *JOSA* 1131, giving a total of 2053. In this way the full material of the individual years was processed, with the exception of the *PHJ* in 1969: only the January material of vol. 177 was examined for this journal which has grown to enormous proportions.

According to the numerical results[15] of this first investigation, which was of both synchronous and diachronous nature, non-indexed eponymal citedness (NIEC) is a very frequent and long-standing phenomenon in the journal literature of physics, with permanent and growing importance in respect of both the number of scientists in question and the number of all eponymal citations not indexed by the Citation Indexes[16]. To mention only the most important numerical result: the non-indexed eponymal reference stock of studied articles of both *PHJ* and *JOSA* amounts to a third of the quantity of the stock of indexed references, for both 1939 and 1969. Furthermore, it was proved that because of the NIEC phenomenon, the producers of really important and long-lasting scientific achievements – continuously many hundreds of prominent earlier scientists – although textually really cited, do not obtain indexed citations of their outstanding achievements. Numerical data of the investigation also indicate that the number of non-indexed eponymal citations in the case of the majority of these prominent eponymous authors not only equals, but often exceeds the number of indexed formal citations of prominent scientists of the present period.

The non-numerical finding of the investigation was that the nature and phenomenology of the NIEC phenomenon, as a conventional form of naming and of referring to long-existing scientific knowledge, were revealed and outlined, showing the origin and development of NIEC. This development already began in the second, declining stage of indexed-formal citedness of the knowledge, when the knowledge was still formally referenced; after this NIEC becomes increasingly frequent, then predominant and finally exclusive. However, by this time the practice of naming and mentioning the knowledge in the anonymous form in the text of articles also generally begins. The anonymous form in reality represents the practice of non-referencing; e.g. 'the second law of thermodynamics' (in the case of a law), or 'as it is well known' (in place of a formula in a mathematical demonstration). Moreover, the final textual form in which long-existing knowledge is mentioned in the physics literature – and also in the literature of other sciences – is now increasingly becoming the acronym, containing either the eponymal or the anonymal designation; e.g. DWBA (= 'distorted-wave Born approximation'), and RI (= 'retention index'). It was found and stated that the emergence of the NIEC phenomenon marks the end of the first stage of the famous Merton 'obliteration by incorporation' as a process. From the viewpoint of indexed-formal citedness, the literature phenomenon of NIEC is a special case of Garfield's well-known 'uncitedness'.

The second fact-finding investigation, which was directed at non-indexed indirect-collective citedness, and the publication of its results is in progress[17] but due to insuperable problems of method, this dealt with only the 1969 part of the first investigation. The number of processed articles was: *PHJ* 331, *JOSA* 290, giving a total of 621; however these were studied with the required philological methods and precision. It was established that non-indexed indirect-collective reference(s) can be found in 7.2% of the articles of *JOSA* – which contains a large proportion (26.6%) of 'Letters' – while the corresponding figure was 14.8% for the articles of *PHJ*, which contains only 2.1% of short communications. However, these incidence ratios are far from expressing the weight and significance of indirect-collective references as non-indexed citations. To determine these, a few normal – non-review – articles containing indirect-collective reference(s) were examined for the number of indirectly cited works and then their ratio to indexed citations was calculated. The result was surprising: the works cited in a non-indexed indirect-collective way in the articles was at least ten times greater than the number of works cited in the indexed-formal way. This means that both the number of scientists who did not receive their citations in the Indexes and the number of non-indexed but real citations was *many times greater* than the number of indexed scientists and the number of their indexed citations. Naturally, the number of all non-indexed – eponymal and indirect – references found in these articles exceeded the number of indexed references to an even greater extent. This surprising result was checked by tests in the material examined for 1939. These checks confirmed that the phenomenon (not only of eponymal, but also) of indirect-collective reference acts existed already in the last, mature period of Little Science. The checks confirmed that in the articles where they existed, the number of objects of such reference acts was also many times greater than the number of formal references which would have been indexed by ISI and would have figured in the Indexes – if they had existed at the time.

As the numerical result of the second investigation it can be stated that in the 1969 material studied, the quantity of non-indexed indirectly-collectively cited works in itself also reaches that of the indexed formally cited works, while the quantity of eponymally and indirectly cited works, i.e. the total non-indexed citations far exceeds that of the indexed ones. This means that in the first decade of the period when both Big Science and the Citation Indexes existed, in the exemplary elite journal literature of the leading science – physics – non-indexed citedness as a literature phenomenon exceeded indexed citedness in frequency.

Until a new, similar investigation covering the current years produces contrary results, the following conclusive statements are justified. The ISI does not take into account and therefore the Citation Indexes do not show the total-real reference stock of processed articles as citations, and so the Indexes do not give a real picture of the work of the scientists who are contributing to the progress of science. Citation data of the Indexes do not fully indicate the actual contribution – recognized really-textually by the publishing scientists – and the quantified data do not quantify the individual extent of this contribution. The failure to take indirect–collective references into account neglects innumerable cases of real recognition of the work of countless contemporary scientists, while the failure to consider eponymal references neglects the impact of the most outstanding earlier creators in the field of science and their genuinely important results which have stood the test of time.

In consequence of the phenomenon of non-indexed citedness, it must (should) be taken into consideration that the stock of indexed references in the journal literature of natural sciences is only *a part* of the real stock of references; that indexed citedness is *not* identical with real citedness; that the Citation Indexes are *not* suitable for the measurement of real literature citedness. There is a need to amend, limit and reduce the now commonly accepted meaning, value and validity of the quantified data of the Citation Indexes and of 'citation analyis' and '-metrics' studies based on them. This need especially applies where these studies wish to (mis)use the citation data of the Citation Indexes for the evaluation of scientists as scientists or other similar, non-bibliographic purposes.

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Human genome studies and intellectual property rights: Whither national interest?

T. R. Sivaramjani and Samir K. Brahmachari

India has been acknowledged as a large reservoir of nature's random mutation, an original 'rich' source of knowledge in the context of international genome studies. Human genome knowledge and the possible understanding of the basis of uniqueness of each individual in chemical terms has presented a number of inescapable challenges to our jurisprudential philosophies and our ethical sensibilities.

Human genome: The new frontiers in science and jurisprudence

'...We really are here on a wonderful threshold of knowledge. The ascent of man is always teetering in the balance. There is always a sense of uncertainty, whether when man lifts his foot for the next step it is really going to come down pointing ahead. And what is needed for us? At last the bringing together of all that we have learned, in physics and in biology, towards an understanding of where we have come: what man is.'

Surprisingly these lines were written not in the present era of genomania but in the early 1970s (ref. 1). The spirit behind it could not ring more true for the science of human genome studies which has been hailed as the last frontier of biology and in what has now become a cliched comparison, has been likened to the Apollo space program. At last we seem to be succeeding in bringing together of all that we have learned in physics and in biology towards an understanding of what man is[1]. The Human Genome initiative is a world wide research effort with the goal of analysing the structure of the human genome at the nucleotide level. It is likely to provide in-depth understanding, and possible treatment, for more than 6000 genetic diseases that affect humankind as well as genetic alterations that increase the risk of developing some common diseases. In addition, the variability in response to pathogenic organisms and the basis of neoplastic proliferation and human behaviour are also likely to be unraveled, thus paving the path of an era of predictive medicine.

A comparison between populations in terms of physiology and susceptibility to certain diseases, whose incidence seems to result from genetic and environmental interactions, would also be of great interest to medical researchers. The vast Indian genetic pool provides a unique opportunity to discover functional significance of human genome sequences of hitherto unknown function through mutation analysis. Thus, India has been acknowledged as a large reservoir of nature's random mutation, an original 'rich' source of knowledge in the context of international genome studies. Apart from the fact that a considerable number of rare genetic disorders are likely to be found merely because of the population size, the large size of most families in India also make them ideal for genetic analysis. The large number of very competent medical practitioners with modern clinical expertise and vast network of hospitals, clinics and health centres, again, unlike in many developing countries, provide conditions conducive to the unraveling of this knowledge.

This human genome knowledge and the possible understanding of the basis of uniqueness of each individual in chemical terms has presented a number of inescapable challenges to our jurisprudential philosophies and our ethical sensibilities[2]. Illustratively, rule of law and concepts of justice are based on the fundamental assumption that all humans are equal. Jurisprudence all over the world has evolved towards taking positive action to make up for social disabilities and allow an opportunity to the socially disadvantaged to also manifest their equality. But genomic research is enabling us to understand that we are different in ways that we could never have understood before. This insight has the political and social potential to shake the very presumption of 'equality' that is the basis of the administration of justice anywhere. Thus in its potential to bring about change, in its capacity for beneficence and also in its latent potential for abuse, the Human Genome project is remarkably similar to the triumph of the splitting of the atom.

Human genome: An emerging economic issue

Although deciphering the human genome as a worldwide effort started as an intellectual and scientific endeavour, now a new generation of the biotechnology industry, the genomic companies, have put in the resources for gene hunting that the academia simply cannot match[3]. The driving force behind this boom is investment from the pharmaceutical industry. Anticipated business out of the genome analysis project and the exploitation of derived knowledge is estimated to be over US $100 billion per annum, starting 2005. Most of it will be for predictive medicine centered around DNA

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based diagnostics and therapeutics. This amount is several fold higher than the world-wide sale of pharmaceutical drugs as curative medicine today. All over the world, legal structures and policy makers have to gear up to face the demands of this new science and its commercial potential. In this context a number of imperatives for Indian scientists and the government become evident to enable the utilization of India's rich genetic wealth for economic and medical benefits for our people[4].

An issue that has come to epitomize these concerns both in terms of the inadequacy of our legal structure as well as of our philosophical discourses is the issue of patentability of the results of such research. Intellectual property rights are supposed to provide recognition and reward for intellectual creativity, and patents are the strongest form of intellectual property rights protection. Although patents have long been familiar to scientists working in the areas of chemistry and engineering, until recently they remained alien to geneticists, biologists, physicians and other academics involved in research in the life sciences. Things changed fundamentally with the advent of modern biotechnology, and especially with relation to various genetic engineering techniques, which blurred the dividing line between basic and applied research. Apart from the domestic, national legal debates that a number of western countries are going through, the issue for India has a number of peculiarities that makes it a little premature for us to talk about patenting of human genetic materials and the products or processes based on them right away.

In the context of human genome studies, patents have brought certain issues into sharp focus, which by their very nature are indicative of larger issues. It would be infructuous at best and to an extent dangerous to view patents in biotechnology as an isolated issue without understanding the trade-related development and other macro-economic issues.

IPR as a trade issue: Recent developments

Until the early 1980s, protection of intellectual property was never considered as an aspect of a trade regime. Nations both developed and developing, recognized that both as a reward and as an incentive for innovation it was necessary to make payments for intellectual property rights, particularly patents. There was much greater emphasis on using patents as a means for seeking transfer and indigenous development of technology and a tool for industrialization. Several developing nations, including India, did not adhere to the Paris convention for the protection of industrial property[5], because they thought that it would come in the way of their industrialization policy[6]. The efforts of the developing countries at trying to bring about changes in the Paris convention to reflect their development needs and priorities turned out to be an unsuccessful attempt.

Furthermore, from the beginning of the 1980s, the major developed countries, particularly the United States, seized the initiative and went about systematically reversing the direction of change from greater flexibility in the national patent systems to take care of varying needs and levels of development, towards a tightening up of such systems. The GATT related agreement on TRIPS was a culmination of this effort[7]. India being a signatory to the establishment of the World Trading Organization including the agreement on TRIPS has to honour the commitment made to the agreement. In many ways the TRIPS is a move away from the dialogue that was uptil then proceeding between the developing and the industrialized world[8]. TRIPS as an instrument of technological protectionism could freeze the present technology asymmetry between developed and the developing countries.

TRIPS seen as a product of international relations appears to hit India hardest in the field of biotechnology. The issue of harmonization of intellectual property regimes in biotechnology is extremely problematic as there is no consensus even within continental or American jurisprudence. Technical objections to the patentability of such innovations, such as the distinction between an invention and a discovery, adequacy of disclosure and obviousness, are routinely made. However, it is the ethical and moral debates about biotechnology especially human genome technological developments, which rage at national and international levels.

In view of the prospects that the progress of the human genome studies will offer for identifying proteins of interest as potential targets for intervention in disease, identifying potential therapeutic proteins and identifying genes responsible for predisposition to particular diseases, the involvement of private funds has become a crucial aspect[9]. Already at the present stage of development of somatic gene therapy and somatic cell therapy in many legal systems (e.g., US and Germany) it is undisputed that the materials involved, e.g., vectors, somatic cells as well as transformed somatic cells are to be treated either as drugs or as biological products and thus patentable under the laws currently in force[10]. It goes without saying that the necessary investment in this area will only be made if patents are able to offer sufficient security for the investors. Already in Europe the relatively lower investments in genomic studies has been taken to indicate the confused state of patentability of the results.

Patentability: The legal debate

The patent issue came to a head with the US National Institute of Health (NIH) patent claims made for cDNA sequences which many in the scientific community felt to be unfairly broad and too numerous[11]. The argument on one side was that filing a patent application for a fully or partially sequenced gene without having the complete biologic information would be tantamount to claiming the rights to all products resulting from the use of the gene, including the products of gene expression, the antibodies to such products and any potential uses for them. While this argument would have limited weight in patent law, the genome community world wide, as well as the industry rejected the idea of having patent on cDNAs without known functions as this would represent an untenable level of monopolization of research results. This exemplifies the concern that indiscriminate patenting may also hinder product development. Patents are important to commercialization in the biomedical field because they provide the exclusivity that encourages industry to invest the resource necessary to bring an invention from the discovery stage through the stages of development, clinical trials, regulatory approvals and ultimately into commercial production. Although India has never been a major player in the International Genome community, the legal debate around patenting is of significance, as it is an acknowledged fact that supra-national patent systems are required to protect innovations of international scope.

It is generally believed that patents should not be granted for mere discovery of that which exists in nature. The leading case in this area, *Diamond vs Chakrabarty* (1980) (ref. 12) where the US supreme court held a strain of human-made, genetically engineered bacteria to be patentable subject matter is still the *locus classicus* as far as patenting of life goes. While holding that the human intervention, amounted to an 'invention' of the bacteria which did not exist in nature the court relied on the legislative history of the law and said that patentable subject matter was meant to encompass 'anything under the sun that is made by man'.

The ethical concerns expressed against genetic research *per se* at that time was a prohibitionist argument that persists to this day. The error with the prohibitionist argument is that it presupposes that all the potential products of biotechnology will carry unacceptable levels of risk. A responsiveness to the potential risk from new technologies is understandable. However a well conceived response to technological change of any kind is one which deals with the potential for risk in a fair and rational manner. Proper risk management enables humanity to reap the benefits of technological change. But the players in the human genome research, being huge multinational corporations (MNCs), which have not in the past shown themselves to be very amenable to any form of control by the nation state system, raise the fear that the rush to make profits may dilute the process of risk management. Further in the absence of any precedent to allow for the common exploitation of the products and technologies of the genome studies, corporate concerns seem to have the floor entirely to themselves. One apparently effective way to control the activities of these corporations as perceived by a number of countries is to put brakes on the patenting of the products of their research.

Even within a well defined legal system there have been discrepancies in the binding nature of various instruments. The fact that certain discoveries are not treated as inventions under the European Patent Convention (EPC)[13], nor the stringent novelty requirements under the EPC has prevented the European patent office from coming out with Examination Guidelines that are conducive to the patenting of such 'discoveries'. Thus as per the patent office guidelines, '... a substance found in nature which must first be isolated from its surroundings and can be properly characterized either by its structure, by the process by which it is obtained or by other parameters, and is "new" in the absolute sense of having had no previous existence, can be patentable *per se* provided the inventor discloses the manner in which to obtain it in a repeatable way[14]'. The patenting of genes and the knowledge based on them in Europe has culminated so far as in March 1995, the European Parliament rejected for ethical reasons the Directive on the legal protection of biotechnological inventions, primarily because under the proposal, patents on isolated human genes and human gene therapy, even germline therapy were in principle allowed[15].

Some of the arguments against patenting have chosen not to draw a distinction between the patenting of human genes and the patenting of human life, contending that the former amounts to the latter. This contention raised in the case of the patent claim for the DNA fragment encoding human H2-relaxin and its precursors[16], was met with the counter that patents covering DNA encoding do not confer on their proprietors any right whatever to individual human beings, no more than do patents directed to other human products such as proteins. It was added that DNA is not 'life' but a chemical substance which carries genetic information, and can be used as an intermediary in the production of proteins which may be useful medically. The statutory basis for an argument that patenting of human genetic material is immoral comes with Article 53(a) of the EPC, which excludes from patenting inventions the publication or exploitation of which would contradict *ordre public* or morality. Since the opposed patent did not offend widely accepted moral standards of behaviour and since there was no clear consensus amongst members of the public that patenting of human genes was immoral, the patent was upheld and arguments under article 53(a) was rejected. While the relaxin case clearly demonstrated the growing difficulties facing the European patent office, and notwithstanding the favourable outcomes for the patentee, problems persist.

The ethical issues against life forms in general often argue on an 'exceptionalism' approach to human genetic material, contending that it has characteristics that make it different from plant and animal genetic material issues for a number of reasons extraneous to the state of technology itself. For example, Switzerland recently introduced its first law regulating biotechnology, including reproductive medicine. The new law prohibits the introduction of non-human genetic material into the human genome[17]. More significantly, the first international convention on Human Rights and Biomedicine, signed by twenty European countries in early April, comprehensively links the dignity and identity of all human beings to the need to prevent abuse of application of biology and medicine[18].

From a scientific point of view it seems even questionable to distinguish genes depending on their source for, 'there is no difference between a yeast gene and a human gene if you observe it in function, if you introduce mutations and make the yeast gene a human gene there is no difference, there is no ethical issue on the human gene that is different from the yeast gene'[19].

All the same there is growing momentum to the idea that human origin is alone a sufficient cause for exclusion from patentability. The concerns reflect a moral and ethical view that borders on the theological and hence the reluctance of the legal system to incorporate such concerns. The picture of the eccentric geneticist going berserk in his lab in the mind of the public does not help either. Any absolutist approach precludes outright assessment of developments on their individual merits. Just because MNCs are the nearest beneficiaries, it will not do for mankind to cheat itself out of the responsible and regulated use of new biotechnologies. Also a prohibition on patenting will force inventors to seek other forms of protection such as trade secrets. And less disclosure can inhibit accountability and development. Obviously less accountability may well result in a greater potential for risk and not less.

Patent law: The emerging contours

The patent law is in a state of flux, and it will take more time for human legal intellect to come to an understanding of what can be done and what cannot which has always been the concern of the law. But certain indications are clear:

- Without patent protection for investments it is going to be difficult to sustain funding in these areas of research. To that extent intellectual property rights have come a long way from providing a reward for intellectual creativity to a form of protection for investments (see Box 1).
- Rather than view patents now as a facilitator for such research, the time may have come to view the law as an essential instrument to regulate and keep such research from going underground.
- The ethical and legal concerns among the public are very real issues that cannot be brushed aside as long as they remain the subject and object of the research. Considering humanity's sake the increasing legal pluralism that is seen needs to be tackled at an international level. However, hesitation by many nations to cooperate fully in view of the moral and ethical implications of such legislation with respect to biotechnology precludes agreements of any substantive depth. While harmonization of patent laws may be inevitable, compromise at the ethical level by individual nations is going to be the major issue.
- At present, opposition to the patenting of human genetic materials is being mounted on two levels. On the first level is the opposition to the patenting

Box 1

Intellectual property

It is instructive to look at the contours of intellectual property protection as incorporated into the International Bar Association (IBA), Bioethics subcommittee of the Law and Medicine Committee, DRAFT International Convention on the Human Genome (23 October 1996). Article VI of the Draft Convention, reproduced here is illustrative of the shift in the fundamental premise of intellectual property protection in the context of genetic research.

Article – VI

1. The Human Genome in its natural state is not subject to private, national or transnational ownership by claim of right, patent or otherwise.
2. Intellectual property based on the human genome may be patented or otherwise recognized in accordance with national laws and international treaties.
3. The collection, distribution and use of human genomic materials and associated information shall be undertaken on a basis which reflects an interest of the original source and of the depositor of the material to an equitable share of the economic benefit of commercialization based upon:

a) Use of the material and associated information ;
b) The relative significance and/or unique nature and/or rarity of the genomic characteristics of the material and associated information; and
c) The original source and depositor's relative contribution to the overall creation and commercial development of relevant intellectual property.

of 'life' which includes microbial, plant, animal and human life. The grounds for these are largely religious or cultural and to that extent constitute a legally pluralistic approach.

- On the second approach, patenting is opposed on the grounds that people from whom genetic material is taken are not likely to receive any financial benefits from it. The opposition arises from past experience in which large corporations have collected genetic material and knowledge from the Third World and from indigenous populations and then used these to develop and patent agricultural and pharmaceutical products without any benefits accruing to the original donors of the material or the knowledge.
- The clear interests of the nation lie in evolving ways and means to apportion the potential commercial and medical benefits to the participating nations, as contributors of genetic material both in the clinical context and in the context of human genome diversity studies.

International scenario: The Indian concerns

Patenting has become an issue in genetic studies in a clinical context. But most of the international flak has been drawn by the patenting of products derived from the genetic material of indigenous people. As of now, as pointed out in the case of *Moore vs Regents* of the University of California[20] a person who takes part in a clincial study may stand to gain nothing from whatever patents that are granted on products derived from their genetic material. In March 1995 the US department of Health and Human services and the NIH staked claim to the human T-cell line of a Papua New Guinean[21]. The patent was granted and maintained after the challenge. The ethical implications of the use of human material samples for clinical research has been the subject of very sophisticated ethical discussions. One of the first principles is the familiar moral precept that the ends do not justify the means. In clinical research, this means that human subjects cannot be seen merely as tools. On the contrary, concerns for the individual subject's welfare and autonomy must take precedence over the interests of science and society[22]. The libertarian individualist concern is manifested in the stress on effective informed consent for participation in clinical research. In cases where the participants are vulnerable groups, like indigenous people where the researcher–subject asymmetry is heightened, the ethical and hence commercial issues get exacerbated. In communal cultures, individual members of a community often do not have the necessary awareness of the implications of participation in an experiment so as to adequately give informed consent. Thus, the vulnerability to abuse of the informed consent procedure has largely rendered it inadequate as an exclusive means of protecting human rights and the welfare of research subjects. Thus in cases of human genetic studies these issues have been raised in a number of international fora by nation-states keen on protecting what they perceive as sovereign rights over their people. These developments make out a clear case for the involvement of national governments in monitoring such studies in their territory. What is required is an accurate assessment of the implication of such studies. Ideally, the vast genetic resource available in India should be used for the benefit of the entire humanity. Indeed, given the potential for benefiting humankind, the balance of the moral argument shifts to asking why human genome studies should not be carried out.

In the case of population genetics and human genome diversity studies, the sources of protest include international NGOs, the world council of churches, and the world council of indigenous people. Among the most vocal critics of the human genome diversity project are environmentalists and groups in developing countries, who are veterans of earlier campaigns to challenge the increasing control over the world's food crops exercised by a relatively small number of large, and mostly western-based agricultural and seed companies. Thus, concerns about the collection of human genetic diversity stems inevitably from similar controversies related to the collection and storage of plant genetic diversity over the past few decades. Patents on these materials will require the payment of royalties which will, in turn, severely limit the access of scientists from poor countries to research carried out by scientists in developed countries. The issue is of primary concern to third world scientists who feel that though their countries may become suppliers of genetic material for research, they may end up having to pay for the products of these research outcomes. In India with her vast scientific manpower there is no reason why such an outcome should be allowed to materialize. The issue is of special concern to the people of India as, given the potential for contributing to the worldwide effort, India is yet to control the access to and use of human genetic data. Possibly the proposed initiative of the Department of Biotechnology to catalyse and support research on the human diversity of India with a view to providing answers to a wide range of questions of biological, medical and anthropological interest[23] will usher in tighter national systems that will not impede ethical research, nor compromise the interests of the people of India.

The common heritage of humanity concept

New international instruments that have been attempted, including the UNESCO Draft and the International Bar Association (IBA) declaration on the human genome

Box 2

The UNESCO Draft declaration on the human genome and its protection in relation to human dignity and human rights:

Article 1 of which states:

The Human Genome is a fundamental component of the common heritage of humanity and needs to be protected in order to safeguard the integrity of the human species, as a value in itself, and the dignity and rights of each of its members.

The IBA DRAFT International Convention on the Human Genome (23 October 1996)

Article 1 of which states:

1. The human genome is part of the common heritage of humankind.
2. Human genome technology shall be developed and used only in full and complete consistency with the common interests of humanity.

have termed the human genome as the 'Common heritage of humanity' (CHH) (see Box 2) which is a well established juristic concept. Certain parts of the earth, sea or outer space and certain properties like the natural and cultural world heritage covered by the UNESCO Convention of 1972 are now considered by international law as to merit protection by the whole of humanity. Thus the CHH concept when applied to the human genome is regarded symbolically as representing the human species and its specific interest to be protected against the dangers created by man himself. But the use of the term is problematic. The same was used in the 1982 law of the sea convention to designate the deep seabed resources. Subsequently the 1994 agreement of the law of the sea makes a mockery of the term in relation to which nations enjoy the most of the resources. Also in the past the CHH argument has been used by the dominant countries for economic exploitation of plant genetic resources. International treaties, regarding natural resources, such as those covering seabed, outer space and Antarctica prohibit the assertion of national territorial claims. Any such 'common ownership' approach, however is objected to by both developing and developed countries because of the current possibility of asserting intellectual property rights over genetic material[24]. Some others have taken the view that the use of the concept would preclude patentability of human genes which at least in the case of the UNESCO declaration is admittedly not the intention[25]. Thus the issue of how to functionalize the concept of CHH in terms of ownership and possession rights remains open.

Institutionalizing protection: The machinery

It would appear best in the interests of the people of India that the government exercise sovereign rights over human genetic material (which to date extends only to plant and animal genetic material under the convention on biological diversity)[26] to the extent that an authority to prevent 'piracy' of genetic material can be identified. Indeed, illegal or unauthorized transfer of DNA from ethnic groups and patients have been reported in the Indian press[27]. With regard to the fact that human genetic research may well be bringing in the new 'genodollars' to the economy, the government would be well advised to identify institutions that can deal with a process of monitoring and study of the material appropriated. As suggested by a team of concerned Indian scientists, rather than simply export DNA or cell lines, a more fruitful line would be to set up modern facilities in the host country and allow exchange of DNA with other countries for standardization of techniques and comparison. Indeed the large scientific community, including geneticists, competent medical practitioners and a network of hospitals and health centres make this a workable proposition in India[28]. It has been further suggested that source material could be managed at central laboratories, and if desired made available to international collaborators with due 'protection' including intellectual property rights.

Any attempt to harmonize patent laws in India will have to be inclusive of giving an appropriate status to the original contributors of the material, which is without precedent in the law of patenting and to that extent is going to be difficult to incorporate[29]. This nonetheless has to be seen in light of what harmonization of patent laws are going to cost us in terms of the price of drugs. Thanks to this, low drug prices, the mainstay of our 'health for all' programme may soon be a thing of the past. If the particular community or the people cannot be identified, the commercial cut will have to be handed over to the government so that at least that particular therapeutic process or product can be made available at affordable price levels. There is need also to explore the possibility of allowing the indigenous group themselves to enter into well informed contracts with corporations for returns to the community not necessarily in the form of royalties or cash payments itself. This kind of a practice finds precedent in various incidents all over the world and legal backing from various soft law agreements internationally[30].

Further it would be a mistake to leave the whole issue of the developments in biotechnology to the judiciary to deal within its inimitable case by case approach. We stand to lose a lot without a clearly laid out policy. For this there is need to generate public debate at all levels. An interdisciplinary study group at the national level may be in the best position to study the developments in various jurisprudential systems and will come out with a legislative proposal at the earliest. Though the perspectives are many and the chances of today's law getting outdated by the morrow are very high, there arise certain clearly identifiable factors that will have to be an essential part of any proposed legislative policy on the subject.

The specifics

- It has been presented that the human body (and its component parts) cannot be regarded as an asset, it cannot be marketed, and hence cannot be a source of financial gain[31]. This is also discussed in the Draft European Convention on Bioethics, Article 11, which states 'The human body and its parts shall not, as such, give rise to financial gain.' There is a crying need to come out with a broad policy statement that the human body *per se* cannot become the subject of direct financial gain. In a third world country where life already seems devalued by poverty such a statement is of the essence.
- International law allows for the identification of ownership of sovereign rights over human genetic material with the government. To functionalize this in as categorical manner as possible, an amendment to the Constitution may be neither misplaced nor mistimed.
- A positive step now is in national interest (notwithstanding the 10-year transitional period allowed by GATT) for rather than view changes in a patent law as creating an opening it should be viewed as a means to regulate the flow of human genetic material from India.
- There is need to redefine our penal laws to make the unacknowledged use of genetic material a crime beyond that of the mere ethical or moral stance.
- The exercise of any such ownership rights will have to be restrained by tenets of the public trusts doctrine. Thus the management of the 'resource' will have to be according to and within the parameters of the interests of the beneficiaries. Identification of the beneficiaries among other ways can be done by demand of detailed patent specification which is both a scientific as well as a legal document.
- The entire exercise should proceed with the full participation to the extent possible of the communities or patients contributing the material. Such 'participation' will have to be at all levels, at the planning, collection, research and commercialization stages, with scrupulous adherence to ethical behaviour and tenets of medical ethics at all times. To make such participation and informed consent truly effective, there is need to disseminate correct information as widely as possible.
- It is necessary to recognize that understanding the human genome is likely to identify some populations which will be susceptible/resistant to certain genetic and environmentally acquired diseases. Therefore, decisive measures need to be taken to prevent any discrimination, legal, social, economic or otherwise.
- The tendency to approach individuals of a particular community or patients in promise of financial gain, is to be discouraged. The gain in terms of intellectual property rights and financial profit should be channelized wherever possible through a network of medical and research centres.
- An institutional mechanism under the appropriate Department will have to be set up much on the lines of the notification of 25 January 1992 (ref. 32) wherein a nodal point for clearance of the transfer of any biological products or materials abroad was sought to be established. Such large-scale monitoring is possible only if the scientific and medical community of this country are taken into confidence and made to feel a part of the entire exercise of protecting national interests.
- To safeguard national interests, it is necessary that all genetic research involving international collaboration be undertaken after formal clearance of the national government. Such 'clearance', would include the right to monitor, and if necessary, proscribe certain types of usage/transfer of genetic material. Automatically private initiative will have to come under the scrutiny of a knowledgeable scientific body.
- In collaborative research, intellectual property rights are to be protected with a majority share of the patent, if any, being held by the collaborating Indian sites and 20% of the benefits[28] accruing from such a patent being used by the individual institutions to develop better services for the population that provided the genetic material.
- In circumstances where the above is not possible, formulation of other comprehensive methods of compensating communities or individuals for their voluntary contributions and cooperation in the research is essential. A 'people of India Fund' at various regional levels may be a possibility. For this the existing network of growing financial institutions may provide an adequate infrastructure at no additional cost. The compensation may take the form of local development work of whatever kind the community opts for.
- In the long term there is a need to catalyse a public debate on the ethical parameter of predictive and preventive genetic medicine issues like confidentiality, informed consent, right to know, and the right to refuse to know. For this pluridisciplinary bioethics committees in our centres of research as well as

our hospitals functioning under broad legislative guidelines are essential.

The problems are many and the solutions are difficult to come by. But as it is said, the only way to win the game is to play it, as per the rules. The only functional option available given the urgency of the matter is to act now in a decisive manner. Anything less would be disastrous for the people of India.

References and notes

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2. Sivaramjani, T. R., Human Genetic Engineering: The John More story, *1 Law and Medicine*, 1996, pp. 96–101.
3. Starting with about $100 million in 1993, by 1996, drug companies had invested more than $1 billion in 'genomics' biotechnology. Thus 'dazzled by the commercial possibilities, venture capitalists, the big drug companies, and Wall Street are all swooning over genomics, pouring millions of dollars into start-ups, the impact of which is being felt from Boston to Beijing' Cohen, J., *Science*, 1997, **275**, 767–775.
4. Brahmachari, S. K., 26th biennial conference, Paper presented at the International Bar Association, Human Genome Treaty Symposium (medicine and the law BP179) October 21 1996, Berlin.
5. Paris Convention for the protection of industrial property (1883); amended latest in 1967. The convention is applicable to industrial property including not only inventions, trademarks, service marks and industrial designs but also utility models, tradenames, indications of source, appellations of origins and repression of unfair competition.
6. In India the question of ratification of the Paris Convention came up for consideration by the Union Cabinet several times – the last occasion being in the early 1980s and each time the Cabinet rejected the proposal for ratification, Dubey, M., *Unequal treaty*, New Age International, New Delhi, 1996.
7. The TRIPS Agreement basically universalizes the levels of intellectual Property Rights protection now prevalent in the developed countries. And the protection of IPRs provided in the agreement will be enforced through the common Dispute Settlement Mechanism of WTO, which provides for retaliation and cross-retaliation.
8. Specifically from the draft Code of Conduct on the transfer of technology negotiated in UNCTAD and the draft Code of Conduct for TNCs negotiated in the UN both of which were designed to require TNCs to transfer technology to developing countries and to function in conformity with the public interest and national development objectives and priorities of the host country.
9. Cohen, J., *Science*, 1997, **275**, 767–775.
10. Strauss, J., Patenting human genes in Europe-Past developments and prospects for the future, 1995, IIC **26** no: 6, pp. 920–950.
11. Craig Venter and colleagues at the National Institute of Health developed a technique for the rapid sequencing of cDNA, which made it possible to detect in a few months thousands of previously unknown genes and to obtain partial sequences. Although the approach to gene identification using cDNA is not new, newly developed instrumentation, powerful computers and advanced robotics have enabled NIH scientists to sequence large segments of expressed genes rapidly. Healy, B., *New Engl. J. Med.*, 1992, **327**, 664–668.
12. 447 U.S 303.
13. European Patent Convention (1977) The grant of an European patent does not result in the existence of a single unitary patent covering the whole of the territories of the designated countries, but rather leads to a bundle of national patents, each being governed by the same provision as a national patent granted directly in the countries concerned.
14. *supra n* 11.
15. The European Union Directive started in 1988 with the clear aim to introduce throughout the member states patent protection standards comparable to those applied in the USA and in Japan. The proposed directive made it clear that the human origin (of genes, proteins and cells, isolated from the body) alone was not sufficient ground for exclusion. The final Draft failed over the issue of ethics and morals linked to the patenting of human genes and human germline therapy. Strauss, J., *supra n* 11.
16. On 18 January 1995, the opposition division of the European Patent Office rejected the opposition filed by the Green Party in the European Parliament and upheld patent no: 11.2149 relating to human H2–relaxin.
17. Campbell, J. J., *Effects of International Trends and Agreements on Biotechnology Patenting*, 10 Canadian Intellectual Property Review, 1993, pp. 129–143. France amended Article 7 of its intellectual property code and declared as unpatentable the human body, its parts and products and the knowledge of the entire or partial structure of the human gene, as such,... as inventions, the publication or exploitation of which would be contrary to *ordre public* or morality.
18. The convention on human rights and biomedicine, among other things, deals with issues of consent, private life and right to information, intervention in the human genome (allows only somatic cell intervention for preventive, diagnostic or therapeutic purposes), ethics of scientific research, organ and tissue removed from living donors for transplantation purposes and prohibition of financial gain from the disposal of a part of the human body. The Convention was signed on 6 April 1997. (TOI p. 9).
19. Dr Bayreuther *supra n* 11, p. 927.
20. John Moore's doctors discovered that his spleen contained blood cells that produced an unusual blood protein that might be used to develop an anti-cancer agent. The cell line (designed the MO cell line) was made the subject of a patent application, granted in 1984. John Moore filed a law suit claiming that his cells were misappropriated and that he was entitled to profits derived from the commercial use of these cells. The potential value of the products derived from the MO cell line could reach several billion dollars, but the California Supreme court ruled in 1990 that Moore has rights to none of it.

    Although Moore had the right to sue his doctors for failing to inform him of the potential commercial value of his cell line, he did not have rights of ownership over his cells after they had been removed from his body.
21. According to the patent application, blood samples were taken from 24 people of the Hagahai. The cell line, the first of its kind from an individual from Papua New Guinea, is potentially useful in treating or diagnosing individuals infected with an HTLV-1 variant virus. Human T-lymphotropic virus type I(HTLV-1) is associated with adult leukemia and with a chronic degenerative neurologic disease. The novel cell line is of potential value in understanding the enhancement or suppression of an immune response to this virus.
22. All enlightened codes and regulations beginning with Nuremberg code and including the Helsinki Declarations and the US federal regulations, appeal to and buttress this principle. see Annas, G. J. and Grodin, M. A., *The Nazi Doctors and the Nuremberg Code: Human Rights in Human Experimentation*, Oxford University Press, 1992. According to US Government regulations for research conducted by government agencies, the 'legally effective informed consent of the subject or the subjects' legally authorized representative' if the subject is the donor of a blood sample is a legal requirement.

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24. see Walden, I., *Intellectual Property Rights and Biodiversity Conservation* (ed. Swanson, T.), Cambridge University Press, 1995.
25. Lenoir, N., Proceedings of 1995 MURS Japan/UNESCO IBC seminars pp. 12–22, The Eubios Ethics Institute, 1996.
26. The objectives of the Convention are stated broadly and include the conservation, biological diversity, sustainable use of its components and the equitable sharing of benefits which arise out of the utilization of genetic resources. This is to be accomplished by access to genetic resources and technology transfer, taking into account all rights over these resources and technologies. The Biodiversity Treaty will become binding on signatories when 50 states have ratified it.
27. *The Economic Times*, Bangalore, 12 February 1996.
28. Chatterji, S., Jain, S., Brahmachari, S. K, Majumdar. P. P. and Reich, T., *Nature Genetics*, 1997, **15**, 124.
29. There are a number of soft law instruments that may be helpful in working out such a space for a compensatory mechanism within the formal TRIPS arrangement itself, apart from inclusion in specific agreements for scientific collaboration. The UNESCO statement of 1996 is very pertinent in this regard. 'The great ethnic diversity and large population of India has provided and will continue to contribute to the knowledge about genomic diversity of the human species. The Indian scientists and those visiting from many nations support the concept that the developing nations and the specific ethnic groups should receive their appropriate share of the economic and commercial returns derived from medical investigations (clinical trials and genetic epidemiological studies) in and from the biological material derived from their population groups.'
30. Posey and Dutfield, *Beyond Intellectual Property: Towards Traditional Resource Rights for Indigenous Peoples and Local Communities*, International Development Research Centre, Ottawa, 1996.
31. Working group paper on population genetics, International Bioethics Committee of UNESCO, 3rd session, Sept. 1995.
32. The Ministry of Health and Family Welfare, Government of India notification dated 25 January 1992 provides general guidelines for the transfer of biological materials outside India of biological products abroad for investigations that can be carried out in India. It has been decided that all proposals for sending such products abroad should be sent to the Director General, Indian Council of Medical Research, New Delhi, who will be the nodal point to clear all such proposals.

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REVIEW ARTICLE

The structure and function of PQQ-containing quinoproteins

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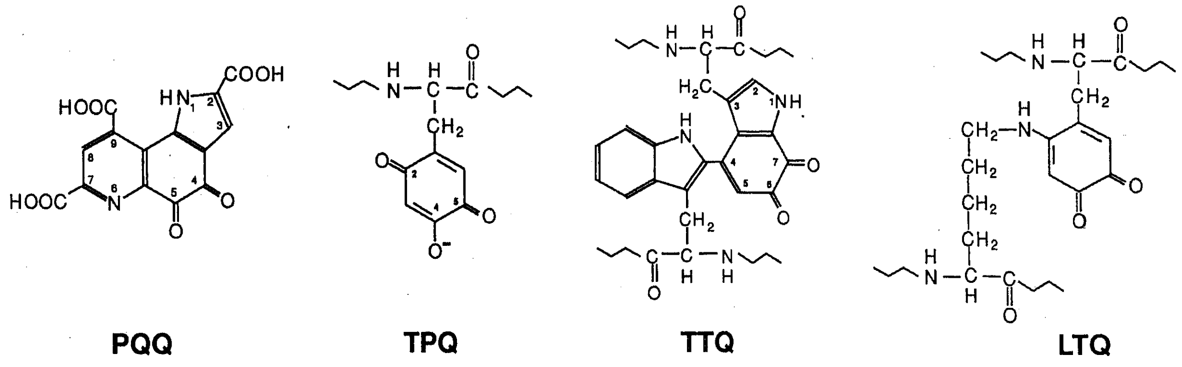
About 30 years ago, it was shown that bacterial methanol and glucose dehydrogenases contained a completely novel type of prosthetic group which was subsequently identified as pyrrolo-quinoline quinone (PQQ). Quinoproteins were originally proteins contaning PQQ but this definition has since been broadened to include those proteins containing other types of quinone-containing prosthetic groups, and the X-ray structures of representatives of each type of quinoprotein have recently been published. This review is mainly concerned with the structure and function of the PQQ-containing methanol dehydrogenase and related proteins. The basic structure is a 'propeller' fold superbarrel structure made up of 8 β-sheet 'propeller blades' which are held together by novel tryptophan-docking motifs. The PQQ in the active site is coordinated to a Ca^{2+} ion and is maintained in position by a stacked tryptophan and a novel 8-membered ring structure made up of a disulphide bridge between adjacent cysteine residues. This review describes these features and discusses these in relation to previously proposed mechanisms for this enzyme.

Introduction to quinoproteins

The term quinoprotein was first coined in 1980 (ref. 1) to include a number of bacterial dehydrogenases which contain pyrrolo-quinoline quinone (PQQ) as their prosthetic group (Figure 1). The name is now used more widely to include all those enzymes whose catalytic mechanisms involve quinone-containing prosthetic



PQQ TPQ TTQ LTQ

Figure 1. The prosthetic groups of quinoproteins. PQQ (pyrrolo-quinoline quinone) occurs in the prosthetic group of dehydrogenases for methanol, higher alcohols, aldose sugars, aldehydes and poly-vinyl alcohol, and for hydroxylation of lupanine. TTQ (tryptophan tryptophylquinone) is in amine dehydrogenases. TPQ (6-hydroxyphenylalanine quinone or topa quinone) is in the copper-containing amine oxidases in bacteria, plants and animals. LTQ (lysine tyrosylquinone) is the prosthetic group of lysyl oxidase, a specific copper-containing amine oxidase occurring in animals.

groups in their active sites. Except for PQQ, these relatively novel prosthetic groups are derived from amino acids in the protein backbone of the enzyme (Figure 1): tryptophan tryptophylquinone (TTQ) is derived from two tryptophan residues and occurs in bacterial amine dehydrogenases[2,3]; topa-quinone (TPQ) is a modified tyrosine residue and is the prosthetic group of the copper-containing amine oxidases found in bacteria, yeasts, plants and animals[4,5]; and lysyl oxidase is a special type of copper-containing amine oxidase whose prosthetic group is lysine tyrosylquinone (LTQ)[6].

The main subjects of this review are the PQQ-containing dehydrogenases, of which the methanol dehydrogenase of methylotrophic bacteria is the best-characterized example[7,8]. The history of the quinoproteins began in the 1960s with the characterization of the novel prosthetic group of this enzyme by Anthony and Zatman[9], and of glucose dehydrogenase by Hauge[10]. More than 10 years later, Duine, Frank and co-workers demonstrated that it contains a quinone structure with 2 nitrogen atoms[11,12], and Kennard's group showed this to be PQQ, by X-ray diffraction analysis[13]. A number of other bacterial dehydrogenases were subsequently shown to contain PQQ by the groups of Duine and Frank in Delft[14], and Ameyama and Adachi in Yamaguchi[15,16]. These quinoproteins usually catalyse the first step in the oxidation of alcohols and sugars in the periplasm of bacteria, thus contributing to the formation of a proton-motive force and hence the formation of ATP[17]. They are usually assayed with artificial electron acceptors such as phenazine ethosulphate (PES). The physiological electron acceptor is a soluble cytochrome *c* in the case of methanol dehydrogenase and some ethanol dehydrogenases; it is protein-bound haem C in the quinohaemoprotein alcohol dehydrogenases, and ubiquinone in the membrane-bound glucose dehydrogenase. For many years it was known that a divalent cation may be involved in the structure or activity of some alcohol dehydrogenases because Mg^{2+} or Ca^{2+} are required for reconstitution of active enzyme from apoenzyme plus PQQ. By contrast, it is not possible to dissociate PQQ from methanol dehydrogenase without irreversible denaturation and it is only relatively recently that calcium has been shown to be present and implicated in the activity of this enzyme[18,19].

Methanol dehydrogenase

This enzyme catalyses the oxidation of methanol to formaldehyde in the periplasm of methylotrophic bacteria and is the only PQQ-containing enzyme for which a structure is available[20-24], the highest resolution structure (1.94 Å) being that of the enzyme from *Methylobacterium extorquens*[23], which is the one referred to throughout this review. It has an $\alpha_2\beta_2$ tetrameric structure; the α-subunit containing the PQQ is 66 kDa, and the β-subunit is very small (8.5 kDa). The subunits cannot be reversibly dissociated and no function has been ascribed to the small subunit. In the tetramer, the two $\alpha\beta$ subunits are arranged with their pseudo 8-fold axes approximately perpendicular to each other, and the PQQ prosthetic groups are separated by about 45 Å.

Structure of the α-subunit

The large α subunit is a superbarrel made up of eight topologically-identical four-stranded twisted antiparallel β-sheets (W-shaped), stacked radially around a pseudo eight-fold symmetry axis running through the centre of

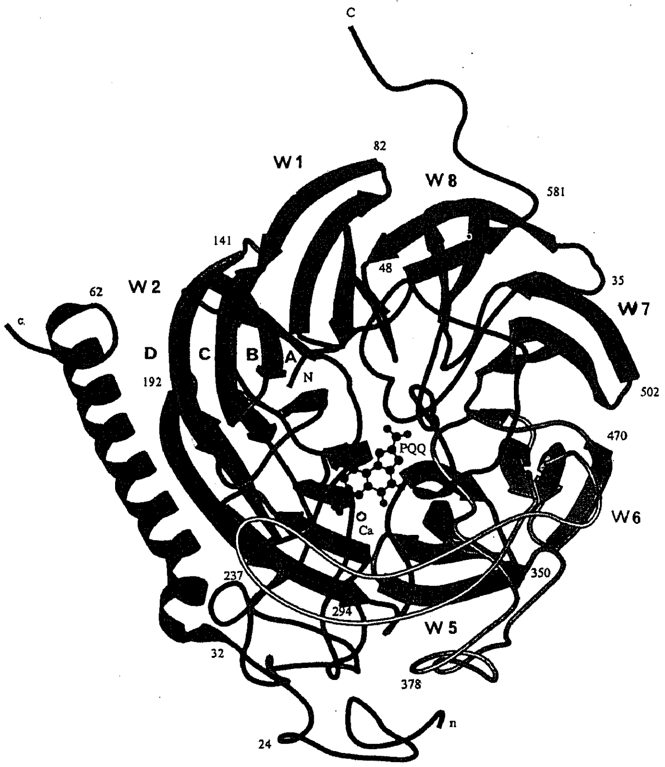


Figure 2. A drawing of an $\alpha\beta$ unit of MDH looking down the pseudo 8-fold axis, simplified to show only the β-strands of the 'W' motifs of the α-chain, and the long α-helix of the β-chain, but excluding other limited β-structures and short α-helices[23]. The termini of the α-chain are marked N and C and those of the β-chain n and c. The PQQ prosthetic group is in skeletal form and the calcium ion is shown as a small sphere. The outer strand of each 'W' motif is the D strand, the inner strand being the A strand. The 'W' motifs are arranged in this view in an anti-clockwise manner. The exceptional motif W8 is made up of strands A–C near the C-terminus, plus its D strand from near the N-terminus.

the subunit. This structure has been referred to as a propeller fold, each W motif representing a propeller blade and examples with four, six, seven or eight propeller blades have been described. Haemopexin[25] and sinovial collagenase[26] have four blades; viral neuraminidase[27] and a related bacterial sialidase[28] have 6 blades; galactose oxidase[29], methylamine dehydrogenase[30] and the β-subunit of the G protein transducin[31,32] have 7 blades, while methanol dehydrogenase and nitrite reductase[33] both have 8 blades. It is intriguing that two of the proteins having the propeller fold structure are quinoproteins, although the related structures have no related catalytic function. Thus, in methylamine dehydrogenase (also from methylotrophic bacteria) the TTQ prosthetic group is in the smaller subunit, the centre of the larger superbarrel being filled with side chains[30]; but, by contrast, in methanol dehydrogenase the active site containing PQQ lies within the large superbarrel structure.

The 32 β-strands that make up the superbarrel structure in MDH are shown schematically in Figure 2, labelled according to the 'W' motif in which they occur (1–8) and the position they take within the motif (A–D). The sequence of the strands in the structure is the same as in the amino acid sequence (this is also seen in other superbarrel structures), with the sole exception that the final strand of the eighth motif (D8) is derived from the N-terminus, not the C-terminus. The short A strands are closest to the pseudo 8-fold axis, and the D strands are on the surface of the subunit. Figure 2 shows that the normal twist of the β-sheet enables space to be efficiently packed in the subunit. This architecture allows the large polypeptide chain to be folded in a very compact form without any other typical structural domains. There is no 'hole' along the pseudosymmetry axis, which is filled with amino acid side chains from the eight A-strands of the β-sheets which are, however, more hydrophilic than those on the B- and C-strands. Strand A runs more or less antiparallel to strand B, but makes only 3 β-type hydrogen bond interactions with it, all of which are close to the A/B turn. It is likely that the conformations of the A-strands are controlled by the close packing of side-chains in the very centre of the molecule, where all eight A-strands come close together; at the point of contact five of the eight residues are glycine, thus facilitating close-packing. The remaining B, C and D strands in each motif form regular twisted β-sheets of sequences of 8 to 9 residues. In all motifs the A–B and C–D corners are short, but the B–C corners are more variable and in three cases (motifs 4, 6 and 8) they form extensions containing 24–30 residues.

Tryptophan–docking interactions in the α-subunit

A series of tryptophan-docking interactions between the β sheet propeller blades make planar, stabilizing girdles around the periphery of the subunits[23,24] (Figure 3). This method of stabilizing protein structures has not been described previously and is therefore described here in some detail. The interactions occur by way of 11-residue consensus sequences in the C/D region of all 'W' motifs except number 8 (Figure 4). The relevant characteristics of the tryptophan residues are their planar conjugated rings, and their ability to act as a hydrogen bond donor through the indole ring NH group (Figure 5). The tryptophan at position 11 is stacked between the alanine at position 1 of the same motif (W_n) and the peptide bond between residue 6 and the invariant glycine at position 7 of the next motif (W_{n+1}). The same tryptophan is also H-bonded between its indole NH and the main chain carbonyl of residue 4 in the next motif (W_{n+1}). The third type of interaction involving the conserved tryptophans is a β sheet hydrogen bond between its carbonyl oxygen

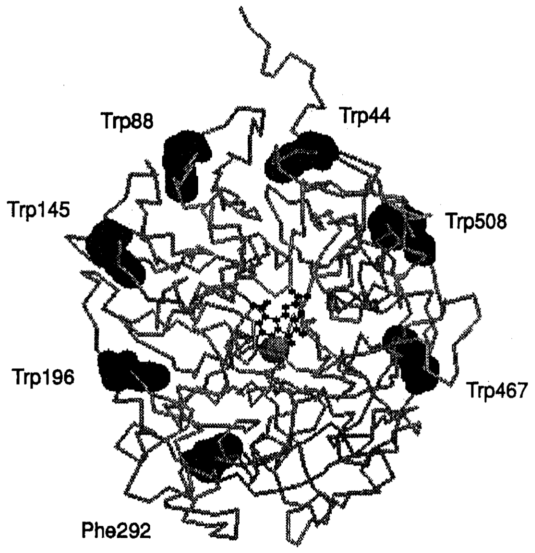


Figure 3. The girdle of tryptophan residues involved in docking the β-sheets together[23]. The tryptophan residues involved in docking are shown in spacefill mode and the rest of the chain as backbone.The PQQ prosthetic group is in skeletal form and the calcium ion is shown as a small sphere.

Position Motif	1 C	2 C	3 C	4	5	-	6 D	7 D	8 D	9 D	10 D	11 D
W1	Ala 77	Leu	Gly	Leu	Asp	Asp	Pro	Gly	Thr	Ile	Leu	Trp 88
W2	Ala 135	Leu	Asn	Ala	Glu	-	Thr	Gly	Glu	Thr	Val	Trp 145
W3	Ala 186	Tyr	Asp	Val	Lys	-	Thr	Gly	Glu	Gln	Val	Trp 196
W4	Gly 282	Arg	Asp	Ala	Asp	-	Thr	Gly	Glu	Ala	Lys	Phe 292
W5	Thr 337	Leu	Asp	Arg	Thr	-	Asp	Gly	Ala	Glu	Val	Ser 347
W6	Ala 457	Tyr	Asn	Ala	Ile	-	Thr	Gly	Asp	Tyr	Lys	Trp 467
W7	Ala 489	Arg	Asp	Ser	Asp	-	Thr	Gly	Asp	Leu	Leu	Trp 508
W8	Val 577	Phe	Ser	Leu	Asp 581	-	Gln 39	Leu	Arg	Pro	Ala	Trp 44
MDH Consensus	Ala	X	Asp/ Asn	X	X	-	Thr	Gly	Asp/ Glu	X	X	Trp
GDH/ADH Consensus	Ala	X	Asp/ Asn	X	X	-	Thr	Gly	Lys	X	X	Trp

Figure 4. The consensus sequences in the tryptophan docking motif[23]. This occurs at the C/D corners at the end of the C strands and the beginning of the D strands of each W motif; there are no loops between these strands. The C/D corners are best characterized as 4-residue (β) turns or 5-residue turns (comprising residues 3–6 or 3–7 respectively). Consensus sequences are also included for the quinohaemoprotein alcohol dehydrogenase (ADH) and glucose dehydrogenase, which is a membrane quinoprotein (GDH).

and the backbone nitrogen of position 1 (usually alanine) of the same motif.

Exceptions to these interactions with tryptophan occur in W1 and W7. In W1 the glycine at position 7 is replaced in the stacking interaction by proline at position 6; and in W7 the alanine at position 1 (Ala498) does not form the expected interaction with Trp508. There are two exceptions to tryptophan in position 11. Tryptophan is replaced by Ser347 in W5 which makes a hydrogen bond with the peptide carbonyl of residue 4 in motif W6. In the other exception, Phe292 replaces tryptophan in W4 and makes only stacking interactions, with the usual glycine peptide bond of W5 on one side, and a second glycine peptide bond in position 1 of W4. Motif W8 is exceptional in having no consensus sequence (except for Trp44) and so there is no glycine to interact with the tryptophan in the previous motif (Trp508); in its place this tryptophan forms a hydrophobic interaction with the side chain of Leu40 and in the previous motif. The carbonyl of this leucine (position 7) replaces the usual carbonyl at position 4 in forming a hydrogen bond with the tryptophan indole NH. The 11-residue motif is extended in many cases by 2 further residues on the C and D strands which are joined by main chain hydrogen bonds[24]. The presence of the 11-residue consensus se-

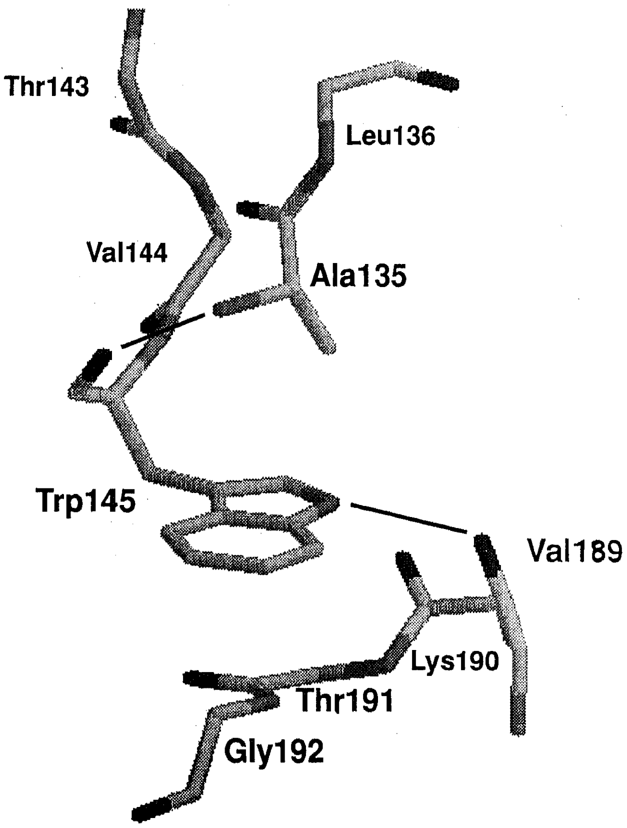


Figure 5. Part of a typical tryptophan-docking motif. This shows the interaction of Trp145 (in W2) with Ala135 (also in W2), and the interaction with the plane of the peptide bond between Thr191 and Gly192 (in W3). For clarity the remaining residues of the two motifs are omitted, and the side chains are omitted from all residues except for Trp145 and Ala135.

quence can most readily be explained if these motifs were derived by divergent evolution from an ancestral 'W' motif containing such a sequence in its C/D corner. This observation adds some further evidence for the evolution of superbarrel proteins which has been considered by Bork and Doolittle[34]. We have observed no similar tryptophan docking motif in the 7-bladed superbarrel proteins galactose oxidase and methylamine dehydrogenase, or in the 8-bladed nitrite reductase, which does, however, have alternative sorts of interactions holding together adjacent propeller blades[33]. By contrast, the PQQ-containing dehydrogenases for glucose (GDH) and alcohol (ADH) show the greatest similarity to the MDH in the sequences which form the W motifs. This has facilitated modelling of the structure of these proteins which has suggested that they have an almost identical tryptophan docking motif at the C/D corners as indicated for MDH (Figure 4).

The α subunits in methanol dehydrogenase contact one another in the region containing the D-strands of the seventh and eighth W motifs, associated over a large planar interface containing many hydrophobic and hydrophilic side chain interactions. This is augmented by the ten C-terminal residues of the α-chains which form extensions which associate with the symmetry-related subunit, again through hydrophobic and hydrophilic interactions.

Structure of the β-subunit

The β subunit (Figure 2) is most unusual as it forms a very extended structure having no hydrophobic core. The N-terminal 30 residues, which include one intrachain disulphide bridge (Cys6–Cys12) and a proline-rich segment (residues 14–20), is folded in a series of open turns. The C-terminal 54 residues, rich in charged residues, form a single straight α-helix of 30 residues in 7 turns followed by a C-terminal segment folded back on the helix. Overall, the β-chain forms a planar 'J' shaped unit, with the long α-helix as its stem, which hooks over the globular α-subunit. Although some hydrophobic interactions occur between the α and β subunits, ion–pair interactions are predominant, as expected from the fact that 40% of the β chain residues are charged. The β chain makes contact with the edges of the W1–W4 motifs of the α chain with ion pair interactions involving Glu148, Glu193, Arg197, Lys236, Glu267 and Glu301 on the α chain, with Lys16, Glu48, Arg50, Arg54, and Lys59 of the β chain. In the absence of any other obvious function for this unusual subunit, it has been suggested that it acts to stabilize the folded form of the large α-chain. The absence of β-subunits in the other PQQ-containing quinoproteins, however, perhaps indicates that it has a more specific (unknown) function.

The novel disulphide ring structure in the active site

Within the α subunit there is a remarkable novel structure derived by formation of a disulphide bridge between adjacent cysteine residues (Cys103–Cys104); the result is a strained 8-membered ring (Figure 6). Although such a disulphide bridge has been proposed to be present in the active site of the acetylcholine receptor[35] and has been seen in the structure of an inactive, oxidized, form of mercuric ion reductase[36], this is the first time that this structure has been seen in an active enzyme. It has been predicted that if such a structure should unexpectedly exist in a protein then a strained ring would be produced in which a normal planar *trans* peptide bond would not be possible, and that a *cis* peptide bond would be more likely[37,38]. In our 1.94 Å structure of the enzyme it is clearly seen that the peptide bond is, as predicted, non-planar, but it is in the *trans* configuration. The ω angle is 145°, giving a distortion from planarity of 35°. All of the other bond lengths and bond angles in the ring are standard values, including the distance between the sulphur atoms (2.06 Å); the distortion in the polypeptide chain within the disulphide ring structure is thus different from that observed in the isolated Cys–Cys dipeptide model compounds[39–41].

The active site

The active sites within each α subunit occur on the pseudo 8-fold symmetry axis, at the end of the superbarrel structure containing the loops between the B and C strands of motifs W6 and W8 which fold over the end of the superbarrel to enclose the active site chamber. Access is by way of a shallow funnel made up of hydrophobic surface residues[42], leading to a narrow entrance to the chamber containing the non-covalently bound PQQ, a bound Ca^{2+} ion, the novel disulphide ring structure and a potential active site base (Asp303). There is no obvious interaction between the two active sites in the two α subunits and the β subunits make no direct contribution to the structure of the active sites.

The PQQ is held in place by way of two non-polar axial interactions, the PQQ ring being sandwiched between the indole ring of Trp243 and the disulphide ring structure (Figure 6). The indole ring is within 15° of coplanarity with the PQQ ring and in contact with it and, on the opposite side the two sulphur atoms of the disulphide bridge are within 3.75 Å of the plane of PQQ. In addition to these axial interactions, many amino acid residues are involved in equatorial interactions with the substituent groups of the PQQ ring system. These are exclusively hydrogen-bond and ion–pair interactions involving residues mostly on the A strands of the 'W' motifs (Figure 7). Although the number of polar groups

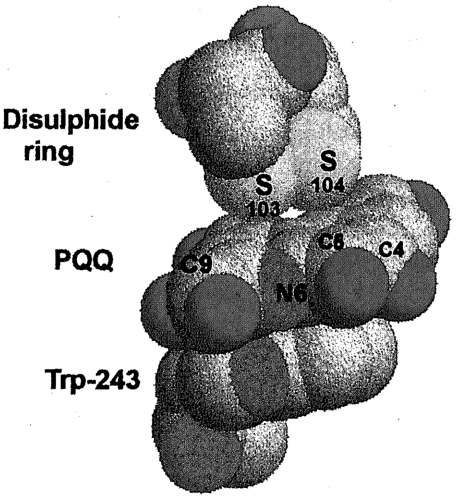


Figure 6. The novel disulphide ring in the active site of methanol dehydrogenase[23]. The ring is formed by disulphide bond formation between adjacent cysteine residues. The PQQ is 'sandwiched' between this ring and the tryptophan that forms the floor of the active site chamber. The calcium ion is coordinated between the C-9 carboxylate, the N-6 of the PQQ ring and the carbonyl oxygen at C-5. The oxygen of the C-4 carbonyl is clearly out of the plane of the ring.

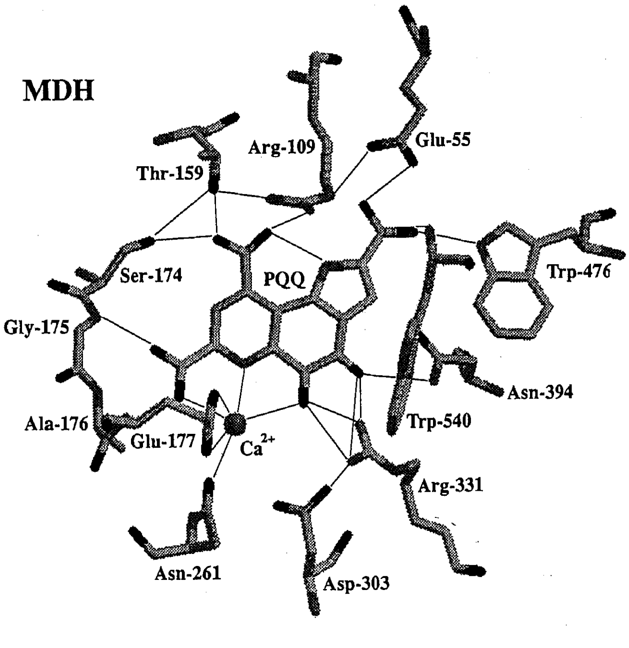


Figure 7. The equatorial interactions of PQQ and the coordination of Ca^{2+} in the active site[23]. This figure also shows Asp303, which is likely to act as a base, and Arg331 which may also be involved in the mechanism. Figure 6 shows the axial interactions that are also involved in holding PQQ in place in the active site.

involved might indicate at first sight that the environment of the PQQ is polar, earlier ENDOR experiments had previously indicated a relatively hydrophobic environment for PQQ and a closer look at the interactions confirms this. An oxygen of the 9-carboxyl forms a salt bridge with Arg109 and both groups are shielded from bulk solvent by the disulphide. The carboxyl group of Glu155 and a 2-carboxyl oxygen of PQQ are also shielded from solvent and it is probable that at least one is protonated, their interaction thus being stabilized through hydrogen bond formation.

Of particular interest are the interactions of the C4 and C5 oxygen atoms; whereas the C5 oxygen is in the plane of the PQQ ring system, the C4 oxygen is out of the plane by about 40° (ref. 23), perhaps consistent with the semiquinone state of PQQ. The C4 and C5 oxygens are hydrogen bonded by the NH_1 and NH_2 atoms respectively of Arg331, and in addition the C4 oxygen makes a longer hydrogen bond interaction with the amide NH_2 of Asn394 whose amide CO is hydrogen bonded to its own main chain NH group. It is not known if the bonding of the C4 and C5 oxygen atoms is maintained in the fully oxidized quinone and fully reduced quinol forms of the prosthetic group, in which the C4 and C5 oxygen atoms are likely to be in the plane of the ring. As well as its hydrogen bonding to the PQQ, Arg331 also makes hydrogen bonds between its NH_2 and the carboxylate and main-chain carbonyl of Asp303. The two side chains lie side by side, permitting free access to the carboxyl group of Asp303, which is the most likely candidate for the base required by some catalytic mechanisms previously proposed for MDH[8].

A Ca^{2+} ion is clearly seen in the active site, confirming predictions that it is likely to be intimately related to the prosthetic group[19]. The co-ordination sphere of the calcium ion in the active site contains PQQ and protein atoms (Figure 7), including both oxygens of the carboxylate of Glu177 and the amide oxygen of Asn261. The PQQ atoms include the C5 quinone oxygen, one oxygen of the C7 carboxylate and, surprisingly, the N6 ring atom which is only 2.45Å from the metal ion. The five oxygen ligands have distances from the metal of 2.4–2.8 Å.

The position of the substrate in the active site has not been unequivocally determined; the one or two solvent molecules which might occupy the same space as the substrate alcohol group are in slightly different locations in the two available structures[23,24]. Immediately adjacent to the more polar region containing the active site base there is a hydrophobic cavity, which could accommodate a small alkyl group, bounded by two tryptophans, a leucine and the disulphide ring. This raises the problem that this enzyme has a broad substrate specificity, and can oxidize primary alcohols including relatively large substrates such as pentanol and cinnamyl alcohol; it is not immediately obvious how these substrates could readily gain access to the active site and understanding of this awaits solution of a structure containing one of these larger substrates.

Mechanism of methanol dehydrogenase

The enzyme catalyses a ping-pong reaction, consistent with reduction of PQQ by substrate and release of product, followed by two sequential single-electron transfers to the cytochrome c_L, during which the $PQQH_2$ is oxidized back to the quinone by way of the free radical semiquinone[12,43–45]. The rate-limiting step is the conversion of the oxidized complex, containing the substrate, into the reduced enzyme plus product, and is the only step requiring the activator ammonia.

The C-5 carbonyl of isolated PQQ is very reactive towards nucleophilic reagents, such as methanol, leading to the obvious conclusion that a covalent PQQ–substrate complex may be important in the reaction mechanism. Support for this has come from the reaction of MDH with cyclopropanol which gives a C-5 propanal adduct, indicating that the mechanism consists of proton abstraction by a base, giving a ring-opened carbanion, which then attacks the electrophilic C-5 of PQQ[46,47]. It was suggested that during oxidation of methanol, a similar proton abstraction must occur, followed by formation of a carbon/oxygen bond to give a hemiketal intermediate. It is probable that Asp303 (Figure 7) provides the catalytic base which initiates the reaction by abstraction of a proton from the alcohol substrate (Figure 8). In the mechanism shown in Figure 7 the oxyanion produced by proton abstraction attacks the electrophilic C-5, leading to formation of the proposed hemiketal intermediate, the subsequent reduction of the PQQ with release of product aldehyde being facilitated by prior ionization of the hemiketal complex which might involve the pyrrole N atom. An alternative mechanism is a simple acid/base-catalysed hydride transfer in which Asp303 again provides the base and Ca^{2+} acts again as a Lewis acid (Figure 9). The large deuterium isotope effect (about 6) observed during the reductive phase of the reaction is consistent with either mechanism; in both cases the step affected will be the breaking of the C–H bond, and it is this step that is affected by the activator ammonia, although the mechanism of this activation is not understood[43,48]. Recent studies using PQQ analogues bonded to Ca^{2+} in organic solvents have provided supporting evidence for mechanisms involving hemiketal formation (Figure 8) (ref. 49).

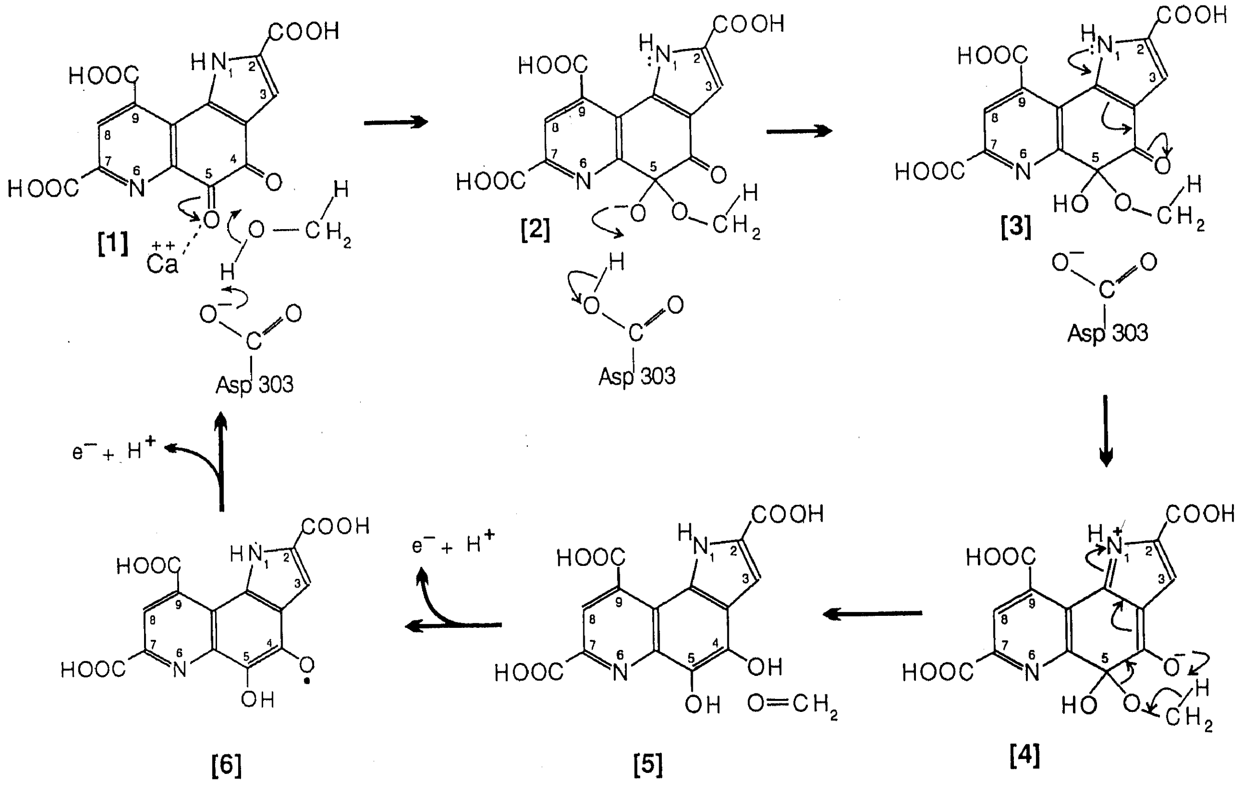


Figure 8. Mechanism for methanol dehydrogenase involving formation of a hemiketal intermediate[8]. It is suggested that the base (Asp303) abstracts a proton from methanol and that the Ca^{2+} ion facilitates attack by the resulting oxyanion on the electrophilic C-5, to give the hemiketal from which the methyl proton is abstracted; this is facilitated by ionization of the C-4 carbonyl oxygen which is made possible by the pyrrole nitrogen atom. The oxidative part of the cycle involves electron transfer to cytochrome c_L or an artifical dye electron acceptor.

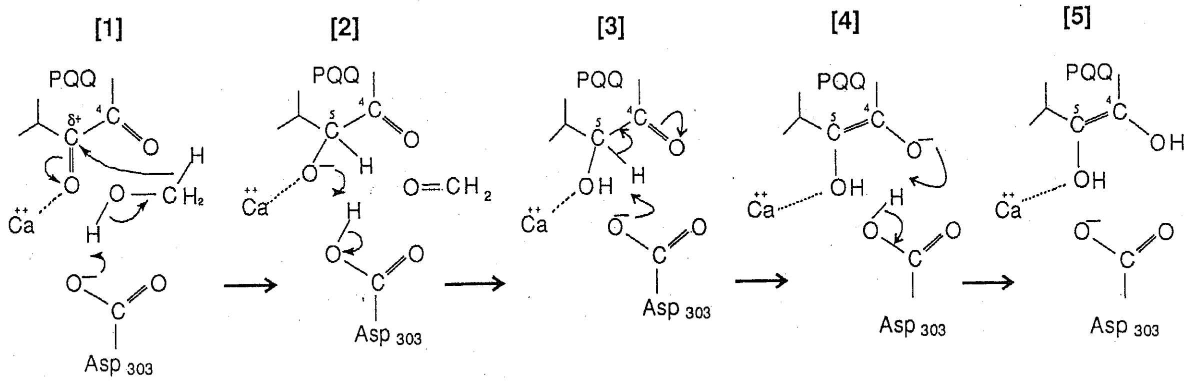


Figure 9. An alternative hydride transfer mechanism[72]. The initial proton abstraction is the same but the electrophilic C-5 is involved directly in removal of the methyl hydrogen as hydride. This mechanism was adapted from those previously published[22] in order to emphase the probable double involvement of the active site base (Asp303). The oxidative phase is the same as in Figure 8.

In the mechanisms described here, the Ca^{2+} ion is given a role in addition to a structural role in maintaining PQQ in an active configuration; it is proposed that the Ca^{2+} acts as a Lewis acid by way of its coordination to the C-5 carbonyl oxygen of PQQ, thus providing the electrophilic C-5 for attack by an oxyanion or hydride[8,42]. It is also possible that the Ca^{2+} ion coordinates to the substrate oxygen atom. The role of Ca^{2+} in the mechanism has been given support by a study of a Sr^{2+}-containing methanol dehydrogenase produced by growing bacteria in a high concentration of Sr^{2+} (ref. 50), and by investigations using an active enzyme containing Ba^{2+} instead of Ca^{2+} (ref. 48). This is the first example of an enzyme in which barium plays an active catalytic role; the modified enzyme has a relatively low affinity for methanol (K_m, 3.4 mM instead of 10 μM), and its activator ammonia, but its activation energy is half (and its V_{max} twice) that of the normal Ca^{2+} enzyme. We have suggested that this may be due to a change in conformation at the active site, leading to a decrease in free energy of binding and hence to a decrease in activation energy[48]. It should be noted that, as an alternative to Ca^{2+} acting as activator of the C5 atom, it has recently been suggested that Arg331, which hydrogen bonds to the C5 oxygen, might be involved as an electrophile leading to build up of positive charge density on this oxygen[24].

Reaction of methanol dehydrogenase with its electron acceptor, cytochrome c_L

Cytochrome c_L is a specific large acidic *c*-type cytochrome[51–53]. There is considerable evidence that the dehydrogenase and cytochrome c_L 'dock' together intially by electrostatic interactions between a small number of lysyl residues on the dehydrogenase and carboxylates on the cytochrome[45,52,54,55]. A study of this initial interaction confirmed the role of electrostatic interactions but surprisingly showed that it is not inhibited by 50 μM-EDTA which is sufficient to inhibit the overall electron transfer process between the proteins[56]. It is possible, therefore, that EDTA inhibits by binding to nearby lysyl residues, thus preventing movement of the 'docked' cytochrome to its optimal position for electron transfer, which probably involves interaction with the hydrophobic funnel in the surface of the dehydrogenase[23,42,56–58].

Electron transfer from the quinol form of PQQ to the cytochrome electron acceptor occurs in two single electron transfer steps – the semiquinone form of PQQ being produced after the first of these transfers (Figure 8). The protons are released from the reduced PQQ into the periplasmic space – thus contributing to the protonmotive force[17,59]. An obvious candidate as an intermediary in this process is the novel disulphide bridge between adjacent cysteines in the active site. This possibility appeared to be supported by the demonstration that this novel structure is very readily reduced with dithiothreitol, yielding enzyme that is inactive with cytochrome. However, reaction of the inactive reduced enzyme with iodoacetate produces carboxymethylated cysteine residues that would not be available to take part in oxidation/reduction reactions, and yet this carboxymethylation leads to re-formation of active enzyme[60]. This type of disulphide ring structure has not been observed previously in an active enzyme and its rarity would suggest some special biological function. It is not present in the quinoprotein glucose dehydrogenase in which electrons are transferred to membrane ubiquinone from the quinol $PQQH_2$, and in which the semiquinone-free radical is unlikely to be involved as a stable intermediate. It has been suggested, therefore, that this novel structure might function in the stabilization or protection from solvent at the entrance to the active site of the free radical PQQ semiquinone in methanol dehydrogenase[60].

PQQ-containing dehydrogenases for alcohols and glucose

There are three types of PQQ-containing alcohol dehydrogenase that are distinct from methanol dehydrogenase[15] but they all contain a calcium ion, and the reductive parts of their mechanisms are likely to be similar. The first type, such as that in *Pseudomonas aeruginosa* is almost identical except for its substrate specificity[61]. The other types are quinohaemoproteins, having an in-built electron acceptor in the form of haem C which occurs on a C-terminal extension of the primary sequence. This is illustrated in Figure 10, which also shows the membrane glucose dehydrogenase with its N-terminal additional sequence likely to be involved in binding the enzyme in the membrane.

The quinohaemoprotein alcohol dehydrogenase from acetic acid bacteria is membrane-bound and contains three types of subunit but no subunit equivalent to the small β subunit of methanol dehydrogenase[15,16]. The primary sequence of the catalytic subunit shows an N-terminal region (600 residues) with an additional C-terminal extension containing a haem-binding site[62,63]. In the N-terminal region there was 31% identity to the sequence of methanol dehydrogenase and it was possible to model its structure using the coordinates of methanol dehydrogenase (Figure 11) (ref. 64). Although there are considerable differences in the external loops, particularly those involved in the formation of the shallow funnel leading to the active site, the active site region was highly conserved, including the tryptophan and the disulphide ring on opposite sides of the plane of the PQQ, and also most of equatorial coordinations to the PQQ (Figure 7). Especially important with respect to the mechanism was the conservation of the active site base (Asp303 in methanol dehydrogenase) and all the coordinations to the calcium ion. This suggests that the mechanism of this alcohol dehydrogenase is essentially similar to that of the methanol dehydrogenase. Comparison of the protein sequence of the soluble quinohaemoprotein ethanol dehydrogenase from *Comomonas testosteroni* leads to a similar conclusion for that enzyme[65].

The membrane-bound glucose dehydrogenase catalyses the oxidation of the pyranose form of D-glucose (at the C1 position) and other monosaccharides to the lactone. The reaction occurs in the periplasm and the electron acceptor is ubiquinone in the membrane[66,67]. It is an intrinsic monomeric membrane protein (about 87 kDa) in which a divalent cation is necessary for reconstitution of active enzyme from apoenzyme plus PQQ. The N-terminal region (residues 1–154) forms a membrane anchor with 5 trans-membrane segments, and this region is likely to contain the ubiquinone-binding site. The remaining periplasmic region (residues 155–796) shows 26% identity of sequence to that of the α-subunit of methanol dehydrogenase and it has been possible to model its structure using the coordinates of methanol dehydrogenase (Figure 11) (ref. 68). In the model structure, the novel disulphide ring is replaced by a histidine residue which maintains the position of PQQ in

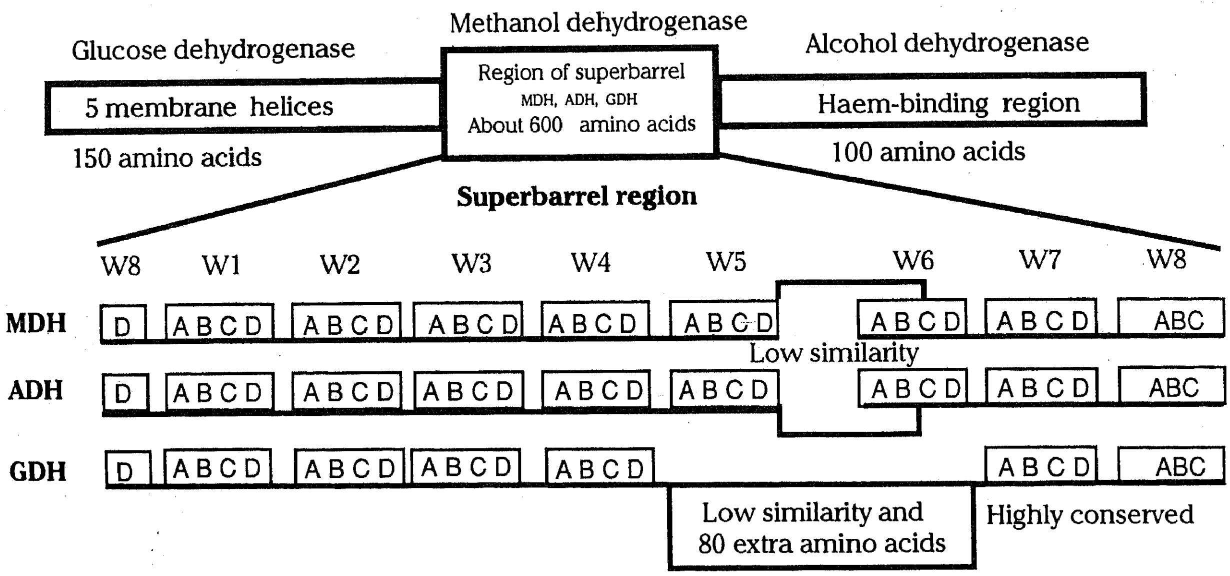


Figure 10. Sequence alignment of quinoprotein dehydrogenases. Each 'W' is a 4-stranded β-sheet (or propeller blade). These are the regions showing greatest similarity of sequence between the quinoproteins. There are many loops between, and within, the β-sheets which show least similarity. For example, there is a long region with little conservation of sequence (including a large loop) between the end of the D-strand in W5 and the end of the D-strand of W6. The highly conserved region between strand-A in W7 and the end of strand-B in W8 was originally proposed to be a PQQ-binding domain; this is not the case.

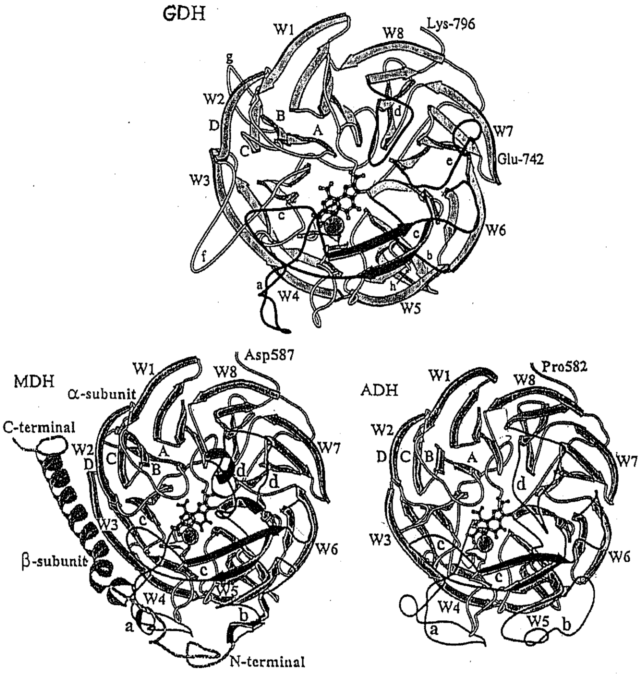


Figure 11. Schematic representation of the backbone of glucose dehydrogenase (GDH), methanol dehydrogenase (MDH) and alcohol dehydrogenase (ADH) showing their major secondary structure. The MDH was determined by X-ray diffraction[23]. The model GDH structure is of the C-terminal section of the membrane-bound GDH (residues 155-796) (ref. 68). The model ADH structure is of the N-terminal region of the quinohaemoprotein subunit I of the membrane complex (residues 1–590) (ref. 64). The prosthetic group is shown as a ball and stick structure, and the Ca^{2+} as a van der Waal's sphere. The major loops are in black. The position marked 'h' shows the position where residues 497–579 (GDH) would join the main super-barrel structure. These residues are not present in MDH or ADH and the sequence on GDH is too long to model.

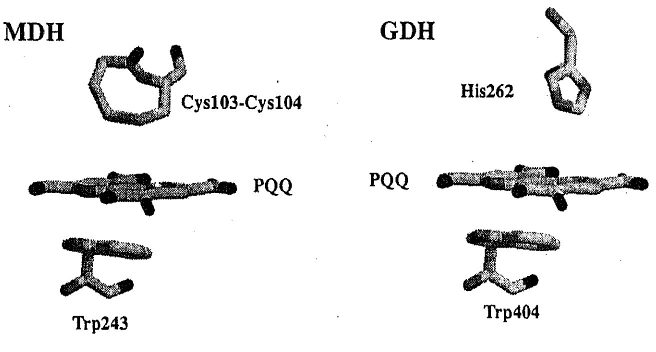


Figure 12. Comparison of the stacking interactions of the PQQ in MDH and the model GDH. In MDH the PQQ is stacked between the coplanar Trp243 and the disulphide ring system of Cys103 and Cys104 (ref. 23). In GDH the coplanar tryptophan is retained (Trp404) but the disulphide is not conserved. Instead, His262 may perform a similar role in helping to bind the PQQ into the active site region. In the model structure the C4 carbonyl oxygen is shown out of the plane of the ring as in MDH, but there is no evidence that this semiquinone structure is ever present in GDH.

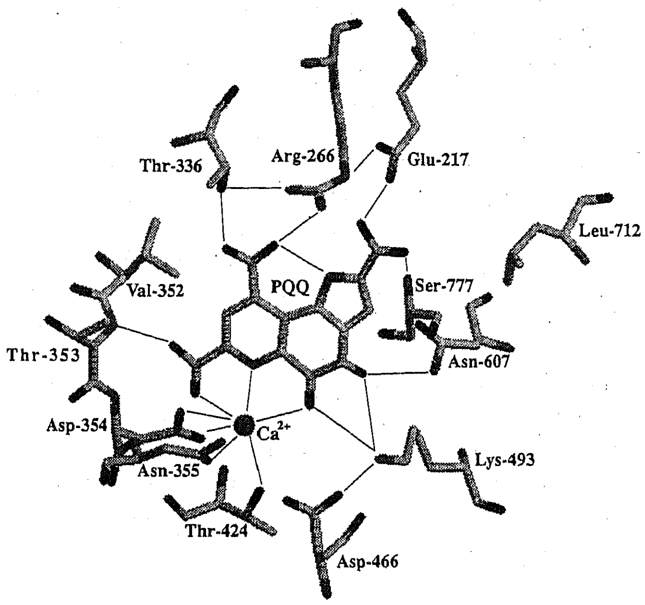


Figure 13. The coordination of Ca^{2+} and the bonding of PQQ in the active site of the model glucose dehydrogenase. Of the equatorial interactions with PQQ the significant differences between the two enzymes are that residues Ser174, Arg331 and Trp476 of MDH are replaced by Val352, Lys493 and Leu712 in GDH; this results in fewer H-bonds to the PQQ in GDH. Ca^{2+} is included in the model GDH, although this may be replaced by Mg^{2+} in the GDH from some bacteria. By analogy with the mechanism proposed for MDH (refs. 7, 8), Asp466 may act as a base, initiating the reaction by abstraction of a proton from glucose; in this mechanism the Ca^{2+} acts as a Lewis acid, co-ordinating with the C-5 carbonyl oxygen, which gives rise to the electrophilic C-5 carbon of PQQ.

the active site, consistent with the previous demonstration that a histidine residue is essential for binding PQQ[69] (Figure 12). There are fewer equatorial interactions between the protein and PQQ (Figure 13), perhaps explaining why it is possible to effect the reversible dissociation of PQQ from the glucose dehydrogenase but not from methanol dehydrogenase[15,70]. By analogy with the methanol dehydrogenase structure, Asp466 is likely to be involved in base catalysis. One clear difference is that there is more 'space' in the glucose dehydrogenase active site, perhaps to accommodate the larger substrate, and the Arg331 in MDH which may play a role in catalysis is replaced by Lys493 in glucose dehydrogenase[68]. The ligation of the (presumed) calcium is similar, suggesting that it plays a similar role in the two enzymes – that of a Lewis acid through coordination to the C-5 carbonyl oxygen, thus providing the electrophilic C-5 of PQQ. The proposed active site base is conserved, suggesting that the reaction is initiated by abstraction of a proton from the anomeric hydroxyl of the pyranose ring. This would be followed by attack from the resulting oxyanion to form a hemiketal intermediate; or attack by a hydride from the glucose oxyanion, leading directly to formation of the lactone and the quinol form of PQQ. That the mechanism might be different, however, is indicated by the fact that Mg^{2+} is far more effective than Ca^{2+} for reconstitution[70,71].

The oxidative half reaction of glucose dehydrogenase is completely different from that in methanol dehydrogenase in which there must be two single electron transfers to two separate cytochrome molecules. By contrast, in glucose dehydrogenase two hydrogen atoms must be transferred to the acceptor ubiquinone. Although this may also involve transfer of the electrons one at a time, it is not necessary for a stable semiquinone to be formed, and indeed no semiquinone has ever been observed in glucose dehydrogenase. The active site funnel is not hydrophobic and there is no suggestion from the model structure or from the primary sequence that there is a hydrophobic region of the protein that could interact with the membrane except the N-terminal transmembrane segments.

Future prospects

Now that the structure of the PQQ-containing quinoprotein methanol dehydrogenase has been determined, the most important remaining questions concern details of the mechanism of its action, including the role of the activator ammonia, the 'docking' system for interaction with its specific cytochrome, and determination of the route for electron transfer within the enzyme and the transfer of electrons to the cytochrome. Similar studies will be extended to the related alcohol and glucose dehydrogenases. The approaches to address these questions involve the analysis of structures of multiprotein complexes, and of proteins containing activator and substrate, and the use of site-directed mutagenesis together with kinetic and structural studies of the mutant proteins.

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A study of source spectrum, site amplification functions, response spectra, Fourier spectra, and peak ground accelerations from the strong ground motion data of the 1991 Uttarkashi earthquake

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The strong ground motions recorded during the Uttarkashi earthquake of 1991 (Ms 7) are analysed for the estimation of source spectrum, site amplification function, decay with distance of peak acceleration and Fourier and Response spectral characteristics. The source spectrum is found to be complex and requires dual corner frequency Brune model to represent it. The high frequency portion of the spectrum, showing the roughness of faulting corresponds to a stress drop of 40 bars, whereas the intermediate to low frequency portion of the source spectrum, related to the overall slip over the fault plane, has a stress drop of 31 bars. The stations in the distance range of 50 km and above show a significant amplification at higher frequencies and the nearer ones like Tehri, Bhatwari and Barkot have amplifications at lower frequencies. The estimate of the site amplification shows an amplification of the order of 3 at some sites. At Tehri the amplification in horizontal components is about 2.5 for the period range of 1.0 to 2.5 s.

THE analysis of near-field of the ground motion gives an opportunity to look into the source processes in finer detail and in establishing relations describing the peak ground motion and response spectra as a function of distance and magnitude in the affected region. These relations are of paramount importance in assessing hazard for the design of engineered structures. The Himalayan region, which has a high seismic hazard potential, has very few strong motion recording sites. The Uttarkashi earthquake of 1991 (Ms 7.0) is the first large earthquake to be recorded at short distances in the Garhwal Himalaya and has provided important database for the appraisal of strong motion expected from a future earthquake in the region. The earthquake inflicted heavy damage, claimed over 2000 lives, left 5000 injured and rendered 10,000 homeless. The economic loss was estimated at Rs 400 crores[1]. The earthquake occurred at 3:45 (IST) in the night hours on account of which the death toll was high. This region was also the site of an earlier earthquake of similar magnitude in 1903 (ref. 2).

We examine here this data set to estimate and model the source spectrum, the site amplification functions, the rate of decay of peak acceleration with distance and the characteristics of the Fourier and the response spectra. We also compare the peak accelerations recorded by structural response recorders and strong motion recorders at some sites.

Seismic regime

Earthquakes in the Himalaya are fed by strain accumulation in the region, which is the boundary between the Indian and the Eurasian plates, that converge at a rate of about 55 mm per year[3]. The great earthquakes that are the principal means of relieving strain occur by thrusting on a sub-horizontal detachment surface[4]. The depth of this detachment surface is approximately 20 km at its northern end. The great earthquakes in the Himalaya in the last 100 years have ruptured sections of the plate boundary in lengths of 250 to 350 km, leaving sectors in between that have not ruptured in the last several centuries[5].

The 1991 Uttarkashi earthquake occurred at the western end of the central gap defined by Khattri and Tyagi[2,5]. This end of the seismic gap is bordered by the eastern edge of the rupture that occurred in the great gap filling 1905 Kangra earthquake[6].

Earthquake parameters

Preferred parameters of the 1991 earthquake estimated from teleseismic analysis are as follows. Estimation of the location also utilized data from the regional and local stations of India Meteorological of Department (IMD).

Origin time: 21:23:14.38 (GMT)
Location: 30.75°N; 78.86°E; $H = 12$ km
$Mo = 1.8 \times 10^{26}$ dyne-cm
$Ms = 7.0$, $Mw = 6.8$, mb = 6.5
NP1: strike 317°; dip 14°; slip 115°
NP2: 112°; 78°; 84°

The nodal plane 1 dipping towards the NW at a low angle is the preferred fault plane as it is consistent with the distribution of aftershocks as well as with the geodynamic model of the region.

The epicenter and the aftershock zone defining the fault area of this earthquake lies in the crystalline rock province of the High Himalaya[7]. The Main Central Thrust follows just on the south of the surface projection of the fault trace (Figure 1). The focal mechanism of the event is also shown in the figure.

Strong motion accelerograms

Strong motion station locations are shown in Figure 1. The figure also shows the surface traces of the Main Central Thrust and the Main Boundary Thrust of Himalaya. Except for Bhatwari, which is situated in the central crystalline province of the Higher Himalaya, the rest of the stations are located in the meta-sedimentary province of the Lesser Himalaya. While Bhatwari is located near the western edge of the estimated position of the fault plane[8], the remaining stations are located quite away from it. The stations may also be grouped in terms of azimuth with respect to the direction of the rupture propagation and the smallest distance from the surface projection of the fault edge. Thus, Bhatwari, Uttarkashi, Barkot, Purola and Koti lie in line with the forward direction of rupture propagation; Ghansiali, Tehri, Srinagar, Rudraprayag and Koteshwar lie in the direction of slip and have an oblique angle with the rupture propagation direction; Karnaprayag, Kosani and Almora are towards a direction away from the direction of rupture propagation. Therefore, on account of rupture propagation the ground motion amplitudes at the first group of stations would be expected to be enhanced, and their frequencies increased due to Doppler shift. On the other hand, the wave amplitudes and frequencies should be reduced for the last group of stations in the rear, while for the second group of stations it should be between the

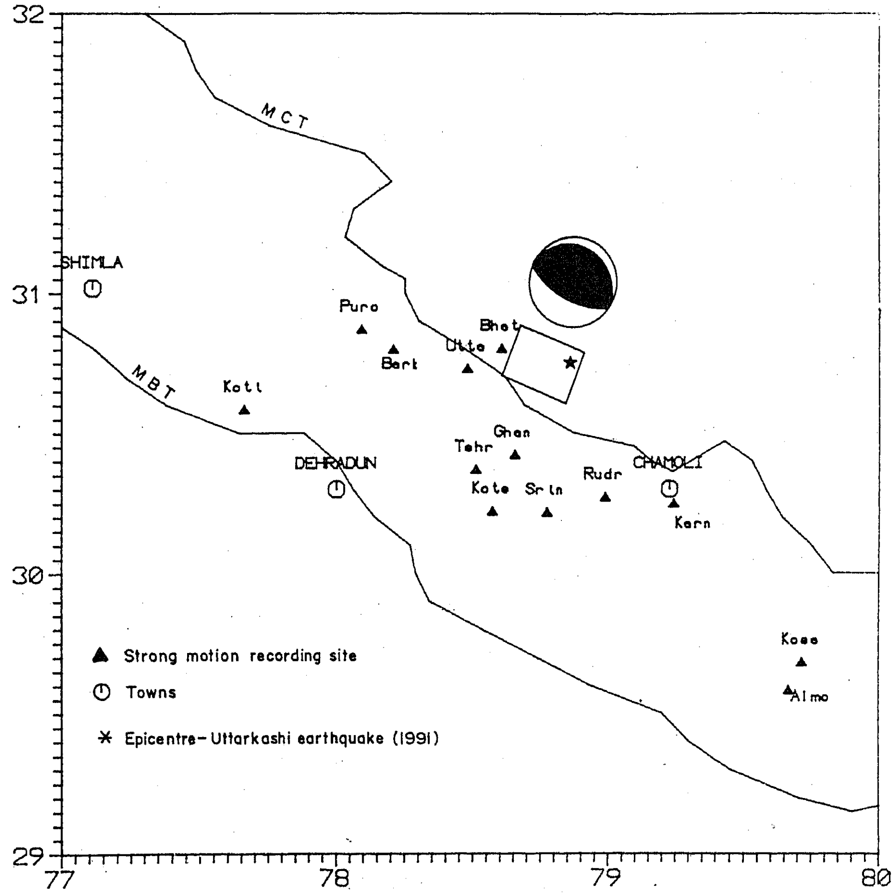

Figure 1. Location of Uttarkashi earthquake (star) and its fault plan solution are shown. The stations are shown as triangles. MBT, Main Boundary Thrust; MCT, Main Central Thrust. The rectangle shows the estimated fault area.

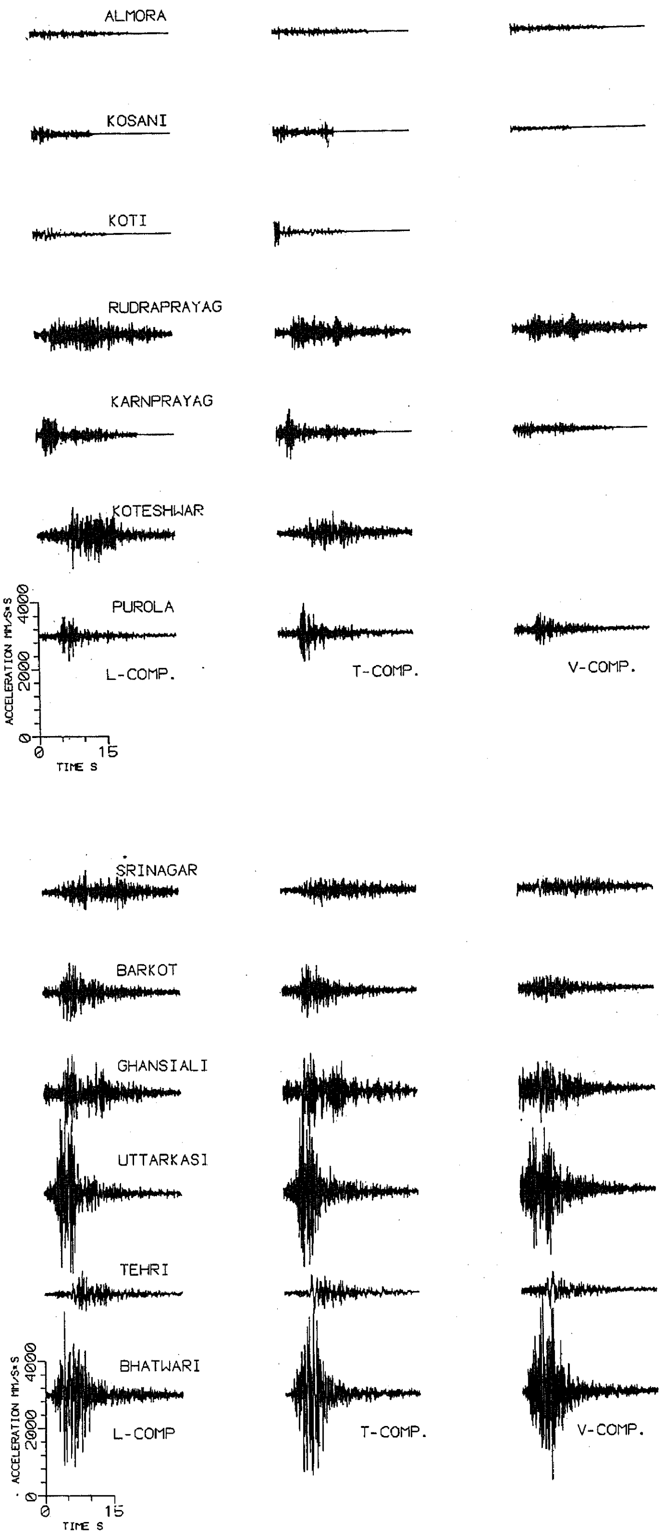

Figure 2. Accelerograms recorded during the Uttarkashi earthquake.

above two ranges. For stations up to a distance of about 60 km, the ground motions are dominated by the direct waves. Beyond this distance the post-critical reflections from Moho and layers within the crust, and *Lg* phases will contribute significantly to the ground motion. There will also be spreading over time of the waves in this distance range.[9]

The strong motion waveforms are shown in Figure 2. These recordings are triggered at some threshold and show the arrival of shear waves starting from where the amplitudes suddenly become large. The data is filtered through a band pass filter (0.17–0.20; 22.0–27.0 Hz). The distances of the stations from the fault edge and the peak acceleration, velocities and displacements are given in Table 1.

The recordings at Bhatwari and Uttarkashi, the two nearest stations show the highest levels of peak accelerations, namely 0.27 g and 0.31 g. We note that another accelerograph at Uttarkashi recorded a peak acceleration of 0.53 g (ref. 10). The waveforms show two bursts of energy spaced by about 2 s. The high frequency signals are overriding the large impulses which have a period of about 1 s. An inversion for the source slip function for this earthquake has mapped several asperities over the fault plane. The two impulses in the records are indicative of the rupturing of the two larger asperities of the fault plane. The durations of strong shaking, defined by a threshold of 0.05 g are approximately 9 s at Bhatwari and 8 s at Uttarkashi (Table 1). The larger peak acceleration observed at Uttarkashi which is at a distance of 10 km as compared to about ~1 km for Bhatwari may be due to local site geology. The Uttarkashi station is in Lesser Himalaya having softer meta-sedimentary rocks and may have a top low velocity layer, whereas Bhatwari is located over crystallines and may lack a significantly thick low velocity top layer. Another factor is that Bhatwari is expected to have a lower amplitude than Uttarkashi on account of the point source radiation pattern of the earthquake.

The stations at Barkot and Purola which are at distances of 32 and 43 km respectively have a relatively richer component of the higher frequencies in the accelerograms as compared to the observed ones at Bhatwari and Uttarkashi. This is surprising as the higher frequencies are expected to attenuate faster than the lower frequencies. The peak accelerations and duration of shacking at these stations have dropped to 0.1 g; 4 s and 0.1 g; 2 s respectively.

The accelerograms at Ghansiali, Tehri and Koteshwar are in the distance range of 26 to 49 km. They record peak accelerations of 0.14 g, 0.07 g and 0.1 g respectively. The durations are 8 s, 2.5 s and 9 s respectively. The accelerograms at Ghansiali and Koteshwar are richer in the high frequencies. There is significant ringing at Koteshwar. The long duration of the signal at this station is on this account. The signals of lower frequencies are especially prominent at Tehri.

The next group of stations are at distances of 45 to 54 km, namely Rudraprayag, Srinagar and Karnprayag

Table 1. Peak ground acceleration *S* and duration of strong shaking recorded during the Uttarkashi earthquake

Station name code	Epicentral distance (km)	Distance from surface proj. of fault (km)	$A_{\max}$ L cm/s^2	$A_{\max}$ T cm/s^2	$A_{\max}$ V cm/s^2	Duration of strong shaking (s)
Bhatwari (Bhat)	25.0	0.0	248.37	241.89	288.78	9.0
Uttarkashi (Utta)	36.0	10.0	237.27	303.99	192.62	8.0
Ghansiali (Ghan)	41.0	26.0	115.59	114.89	99.23	8.0
Tehri (Tehr)	54.0	39.0	71.41	61.13	57.82	2.5
Barkot (Bark)	63.0	32.0	93.18	80.47	43.65	4.0
Srinagar (Srin)	60.0	45.0	65.44	49.45	33.09	1.5
Rudraprayag (Rudr)	54.0	44.0	52.29	50.76	44.13	9.0
Koteshwar (Kote)	65.0	49.0	98.85	65.23	74.34	9.0
Karanprayag (Karn)	67.0	54.0	60.99	77.35	25.96	2.5
Purola (Puro)	75.0	43.0	73.95	91.68	51.74	2.0
Koti (Koti)	116.0	89.0	20.64	40.95	14.25	1.5
Kosani (Kosa)	144.0	144.0	28.34	31.50	11.04	1.0
Almora (Almo)	150.0	150.0	17.41	21.02	18.44	0.5

which are located in the opposite direction of rupture propagation. The peak accelerations and duration of group shaking are 0.06 g, 9 s; 0.06 g, 1.5 s; and 0.08 g, 2.5 s respectively. The records at Rudraprayag and Srinagar display a considerable amount of high frequencies. Karnaprayag has lower frequency content. The long duration observed at Rudraprayag appears to be a case of ringing, perhaps in a shallow low velocity layer. The peak accelerations at Srinagar and Rudraprayag are lower as compared to at Purola. This is interpreted to be due to the directivity effect of the rupture propagation. These stations are at similar distances, but Purola is in the direction of rupture propagation while the other two stations are in the opposite direction.

The remaining three stations are Koti, Kosani and Almora at distances greater than 100 km. The ground motions at these sites will be dominated by the *Lg* phase. The acceleration levels are quite small and of relatively lower frequencies.

The wave packets carrying most of the energy at the stations in the distance range of 30 to 55 km are more dispersed than at Bhatwari and Uttarkashi. The higher frequency content is likely to be due to the *P* waves coming in as wide angle reflections from the top of the basement which is expected at a depth of about 17 km (ref. 2). The direction for such waves is near maximum for the radiation pattern of the earthquake.

Attenuation of peak ground acceleration

The values of the peak ground acceleration at various stations are plotted as a function of epicentral distance in Figure 3. The decay is faster at distances less than 50 km and steady after that. The attenuation relations for peak ground acceleration with epicentral distance[11–14], are also shown for comparison. McGuire's relation overpredicts the expected ground acceleration

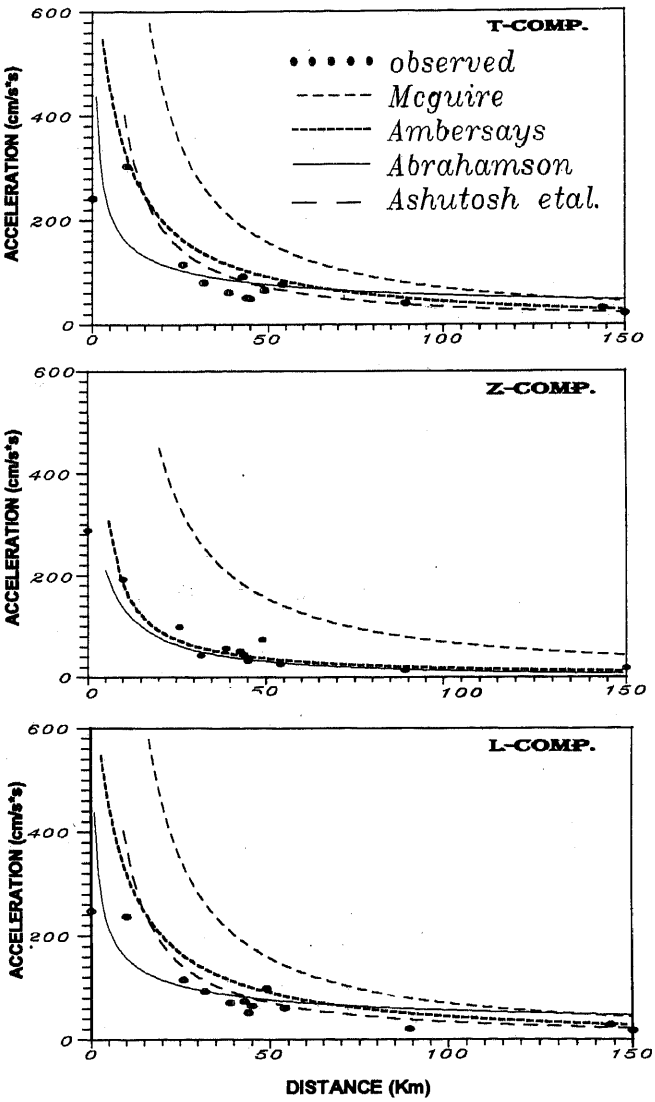

Figure 3. Dependence of peak ground acceleration with distance. See text for regression.

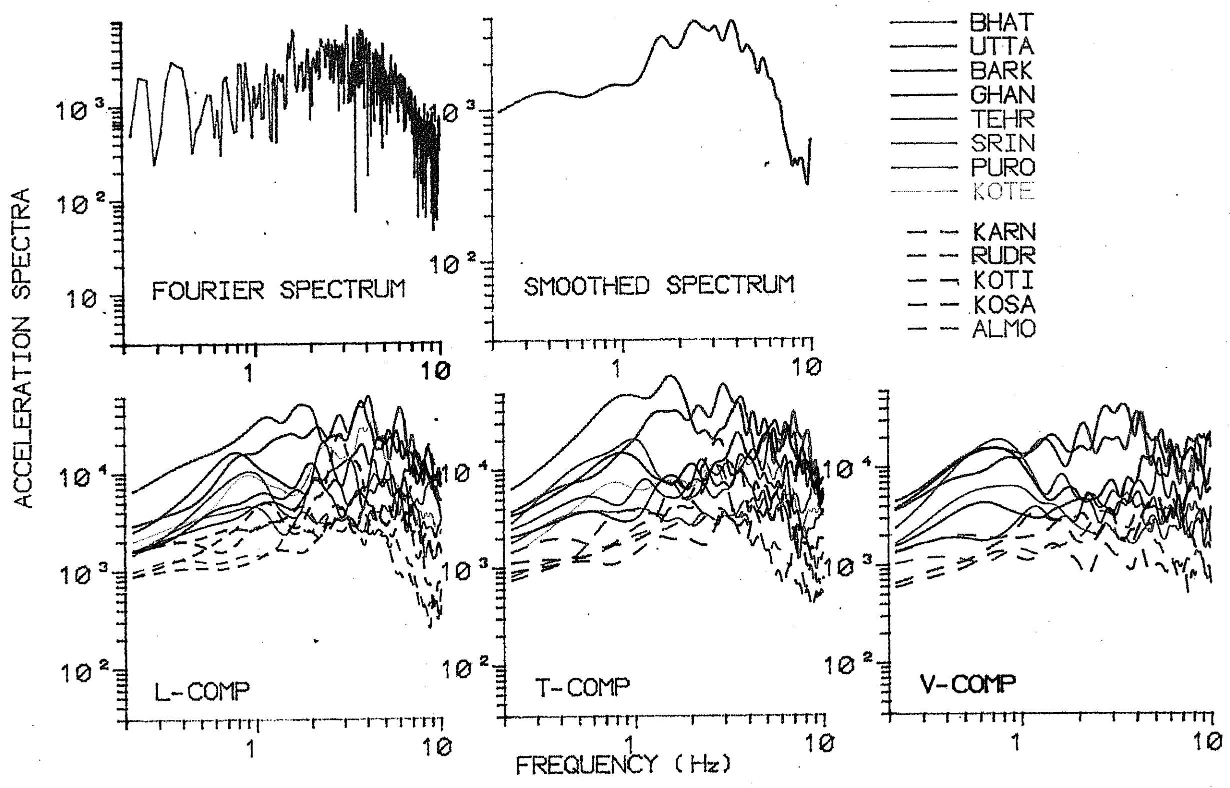

Figure 4. Fourier spectrum of the accelerograms. The top panel shows the spectrum obtained using FFT and the corresponding smoothed spectrum for the station ALMO (L-Comp). The bottom panel shows all the smoothed spectra.

in all the three components. Ambrasey's relation which is based on the European data set matches the observed peaks in vertical component. For the horizontal components the match is good for distances greater than 50 km. Abrahamson and coworker's relation based on data from different parts of the world, shows a better fit in the vertical component but overpredicts at short distances for the horizontal components[13]. Ashutosh *et al.*[14] used data from various Himalayan earthquakes (including this event) in their regression for attenuation relation. This relation gives a better match at short distances up to 50 km but the predicted peak accelerations are higher than the observed ones for the distant stations. This could be due to the inclusion of data set from the Shillong Massif region of NE Himalaya where some of the earthquakes are of intermediate depth and the observed attenuation is unusually lower than in other parts of Himalaya. The inadequacies in representing the decay of peak acceleration by these regression relations emphasizes the need for a localized database to model the attenuation properties of the region.

Fourier spectra

The Fourier spectra of all the recordings have been estimated using Tukey–Hamming technique. Figure 4 shows a spectrum obtained using the fast Fourier transform algorithm. This estimate is quite rough. The corresponding smoothed spectrum is also shown. For the remaining recordings only the smoothed spectra are shown. The spectrum in general shows a hump at 1–5 Hz band, falling off both at the lower as well as at the higher frequencies. The spectra for Bhatwari and Uttarkashi show a maximum in the frequency range of 2 to 4 Hz. The amplitudes fall at higher frequencies. The spectra for the *T* horizontal components at Bhatwari and Uttarkashi are richer in lower frequencies than the *L* horizontal as well as the vertical components. There is more energy in the higher frequencies in the horizontal components at Uttarkashi than at Bhatwari. This is consistent with the higher peak horizontal acceleration, a mainly high frequency phenomenon, observed at Uttarkashi.

The spectra at Ghansiali, Tehri and Koteshwar have relatively larger energy in the 0.6–0.9 Hz frequency range. The Tehri horizontal components are depleted in higher frequencies. The spectra at Rudraprayag have a peak at around 8 Hz indicative of resonance observed in the accelerogram. Similarly the horizontal components at Srinagar have a peak at this frequency. The Srinagar vertical also shows a hump in the 0.5–0.9 Hz range, corresponding to the distinct low frequency pulse in the

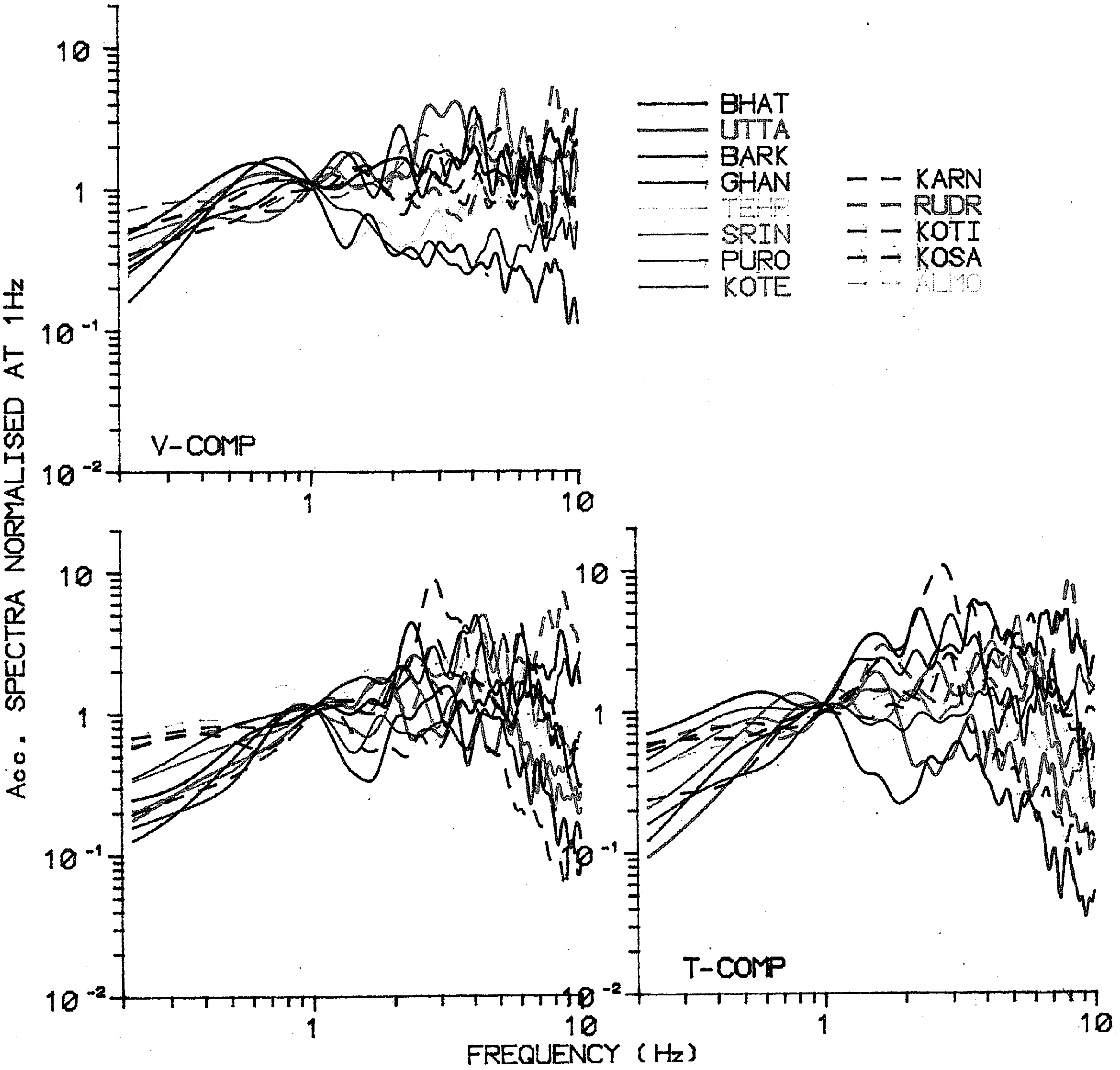

Figure 5. Fourier spectra of the observed accelerograms normalized at 1 Hz.

corresponding accelerogram. The horizontal components have a peak around 2–3 Hz corresponding to the resonance seen in the accelerograms. The vertical component is relatively flatter, with enrichment in the 4–6 Hz band. The spectra at Kosani and Almora have similar structure up to about 2 Hz, with departures seen at the higher frequencies.

In order to compare the relative distributions of the energy in various frequencies at different sites, the spectra normalized at 1 Hz are plotted in Figure 5. The station nearest to the fault at Bhatwari is rich in frequencies less than 1 Hz in the horizontal components. The stations at Uttarkashi and Ghansiali which are 10 to 25 km respectively from the rupture zone, show a faster decay of spectral amplitude for frequencies higher than 1 Hz. Tehri shows a maximum around 0.8 Hz in both the horizontal and the vertical components. Barkot which is at a distance of 20 km from the fault in the direction of rupture is poor in frequencies less than 1 Hz and rich in high frequencies. Thus the spectra in general is dominated by the local site effects rather than the directionality of the fault rupture and other source conditions.

Source spectrum

The spectra at Bhatwari and Uttarkashi, which are at distances very close to the edge of the fault, may be used to estimate the source spectrum. The average source spectrum obtained in this way is shown in Figure 6. This represents the spectrum at the surface and will

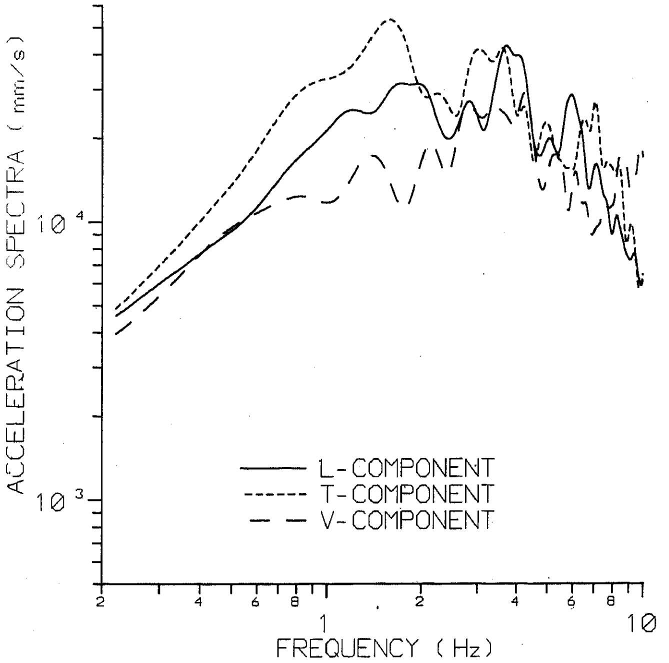

Figure 6. Average acceleration Fourier spectra to reprcsent the 'source spectrum'.

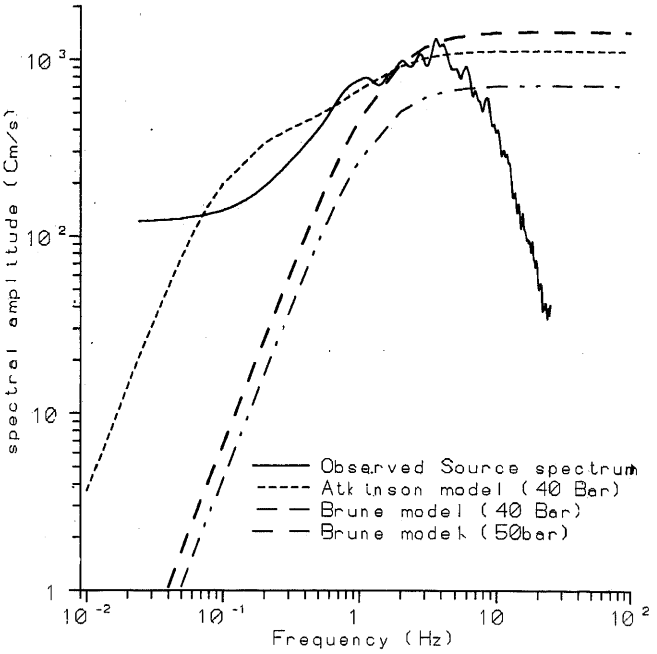

Figure 7. Observed source acceleration spectrum and the corresponding two Brune spectra composite model and the simple Brune model.

suffer from attenuation in the medium due to propagation. The corner frequency is expected to be at about 0.08 Hz and is outside the pass band of the accelerograms and so cannot been seen. On the other hand, we notice a corner at about 1 Hz. The source spectra of many earthquakes have been found to be characterized with a high frequency level with an attendant corner frequency and a low frequency level and corner frequency related with the moment of the earthquake. The spectra at the high frequencies is related to the roughness of the faulting process. Whereas the long period level and the lower corner frequency are related to the main smooth source[15]. The heterogeneous nature of faulting represented by the above character of the source spectrum has been modeled by multiple events[16,17], the specific barrier model[18], the asperity model[19,20] and the partial stress model[21]. More near-source earthquake records will be needed for establishing it. In the Brune model[21] commonly adopted for representing the source spectrum the high frequency level is governed by the stress drop and the low frequency level is proportional to the seismic moment. The spectrum here departs from the basic Brune model. The spectrum is depleted in intermediate frequencies. We do not know whether the observed depletion in the middle frequencies noticed in the Uttarkashi earthquakes is typical of the region. This may be due to the reasons mentioned above and also due to the model assuming a circular fault shape whereas in reality it may be rectangular or irregular in shape[22–24]. Atkinson[15] has found that such spectra for the horizontal component can be adequately modeled as a superposition of two Brune spectra having the following form.

$$A(f) = C(2\pi f)^2 M_0\{(1-\varepsilon)/[1+(f/f_{02})^2] + \varepsilon/[1 + (f/f_{01})^2]\},$$

where $C = R\pi FV/(4\pi\rho\beta^3 R)$ with $R = 1$ km, R_p average radiation pattern (= 0.55), F = free-surface amplification (= 2.0), V = partition of two horizontal components (= 0.71), ρ = the crustal density (= 0.28 g/cc), β = shear wave velocity (= 3.5 km/s) and ε is the moment partitioning parameter ($\log \varepsilon = 2.52–0.637\, M$ with M = magnitude). f_{01} is the upper corner frequency and f_{02} is the lower corner frequency. In the present case the lower corner frequency is estimated on the basis of fault dimension from the aftershock data. The corresponding stress parameter is 31 bars. The higher corner frequency is at 1.3 Hz. From the roughness spectrum envelope, the stress parameter obtained is 40 bars. The corresponding two corner source model is also shown in the Figure 7. The match between the empirically estimated spectra and the model is satisfactory in the 0.4–4.0 Hz band. At higher frequencies the drop in the observed spectrum is due to the attenuation in the propagation and perhaps also due to source properties. Towards the lower frequencies the mismatch could be due to the filtering applied in the conversion of the analog data to the digital mode.

Site response functions

The relative site response functions have been estimated by finding the average spectra for groups of stations that

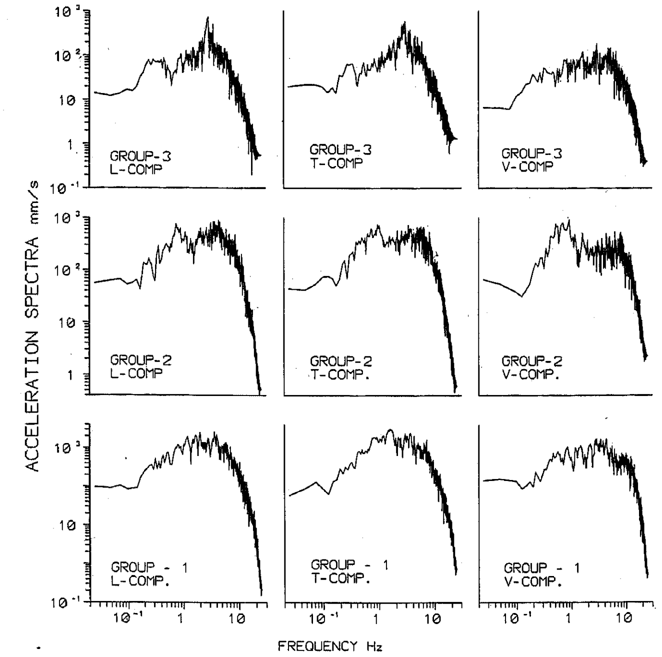

Figure 8. Average acceleration Fourier spectra for groups of stations. Group 1 stations, Bhatwari, Barkot, Uttarkashi, Purola and Koti; Group 2 stations, Ghansiali, Tehri, Koteshwar, Srinagar and Rudraprayag; Group 3 stations, Karanprayag, Kosani and Almora.

are at similar azimuth and distance from the fault edge. Three groups were formed and their average spectra are shown in Figure 8. One notices the consistency of the shape of the spectra. However, there is an enrichment in the frequency band of 0.5–1.0 Hz in the group of stations (including Tehri) on the SE of the fault plane. These average spectra were used to divide through the observed spectra at various stations to obtain estimates of the site amplification functions which are shown in Figure 9. The base line adopted for estimating the amplifications is the low frequency level in each function. In general the amplification bands are in the higher frequencies. We find that in most cases the amplifications are within a factor of 2 except at stations at Rudraprayag, Koteshwar and Karnaprayag where narrow band strong resonances at higher frequencies are present. However, at Tehri there is amplification by factors of about 1.5–2 in the middle frequency band of 0.4–1.0 Hz. This may be contributed by a shallow low velocity layer as well as by the topographic effects. Uttarkashi also shows an enrichment at higher frequencies.

The foregoing re-emphasizes the role of the local site conditions in significantly modifying the ground motions. Here, we see in particular, that the horizontal ground motions will be amplified by a factor of up to 2.5 in the band of frequencies that cover the natural frequencies of the Tehri dam, thus gravely enhancing the seismic hazard of the structure. This aspect has not been

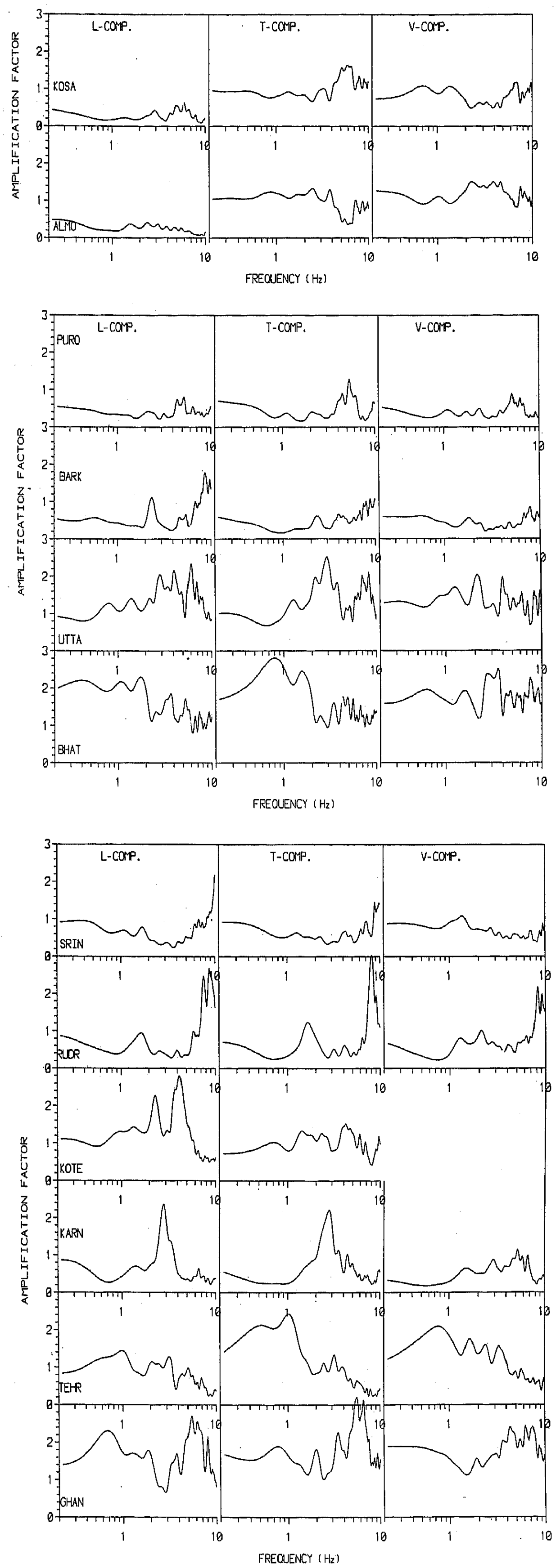

Figure 9. Site response spectra for the ground acceleration.

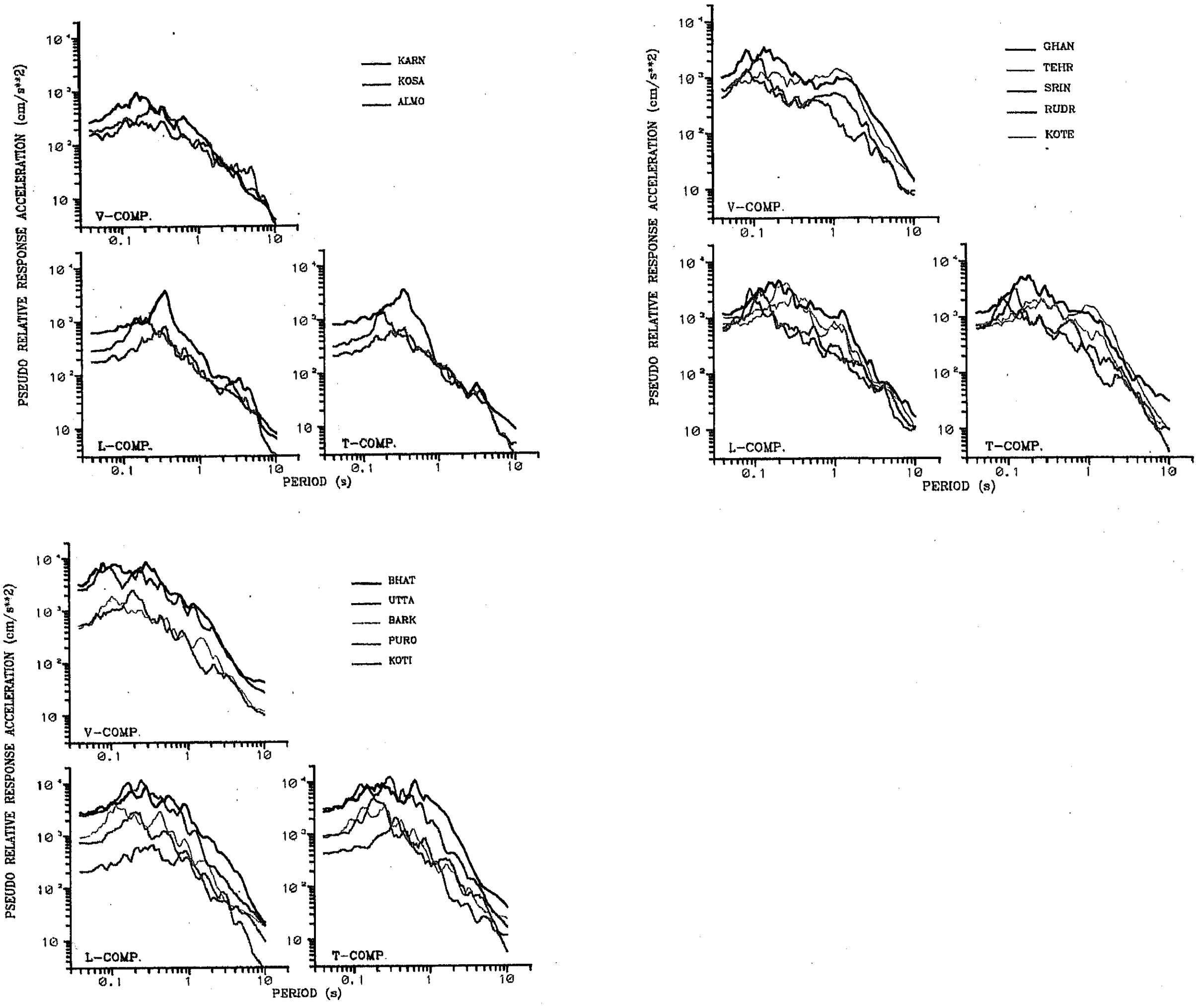

Figure 10. Response spectra for 5% damping for all the sites.

taken into account in the currently adopted design specifications of the dam.

Response spectrum

The response spectra (5% damping) for all the stations are shown in Figure 10. A comparison of the response spectra at Uttarkashi and Bhatwari shows that the amplitudes are considerably larger at longer periods at Bhatwari, whereas they tend to converge at periods shorter than about 0.6 s. Similarly, the response spectra for Tehri also show higher response values at periods longer than about 0.6 s. This is reflective of the dominance of longer periods in the accelerograms at these stations. Hence if we normalize the accelerograms from stations at various distances and average the corresponding response spectra to obtain an average response spectrum, we are going to get quite an inadequate picture for sites such as Tehri as the average response spectrum will be lacking the enhanced response at the longer periods.

To appreciate the extent of enrichment in the energy in the longer periods at Tehri and the nearby station at Ghansiali, their response spectra are shown in Figure 11 along with those of Bhatwari and Uttarkashi which are very near to the fault edge. The response spectra come closer together for all the four sites for periods greater than about 1 s for the vertical component while only Bhatwari is somewhat higher in the horizontal components. This shows the amplifying effect of the local geology at Tehri and Ghansiali. On the other hand, the response spectra diverge at shorter frequencies as is expected due to the attenuation effect of distance from the fault.

Attenuation of response spectrum with distance

The dependence of response spectrum on distance is shown in Figure 12. The dependence is plotted for four periods, that is 0.4, 0.75, 1.25 and 2.0 s. A significant

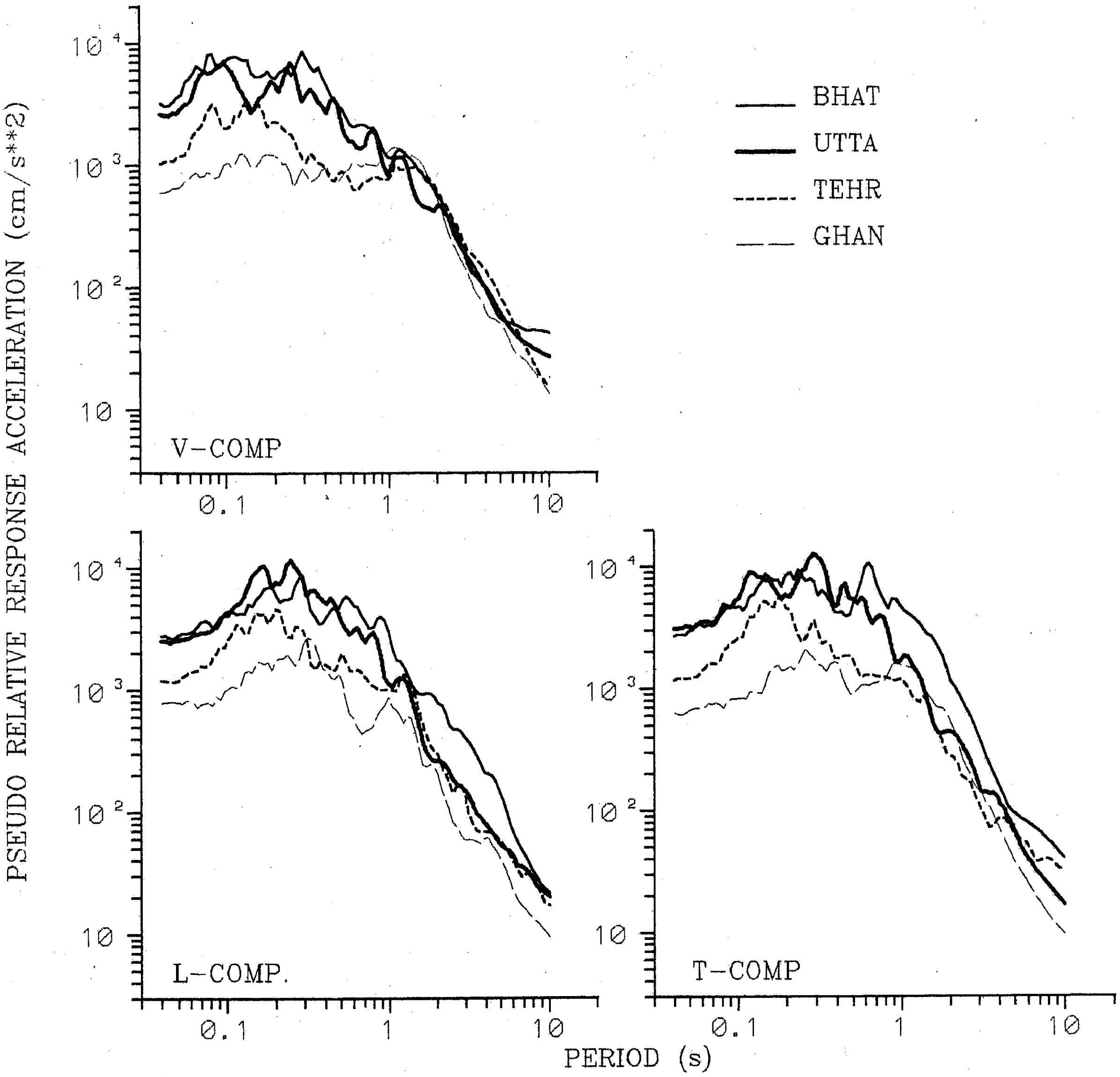

Figure 11. Comparison of the response spectra at Bhatwari, Uttarkashi, Tehri and Ghansiali.

observation is that the group of stations in the 40–50 km distance range generally show anamolously high amplitudes. The amplifications are more pronounced in the horizontal components and in the shorter periods. Tehri, Koteshwar, Ghansiali and Karnaprayag are among this group of stations. This phenomenon is a site characteristic. It may be further examined by investigating the local site geology, and by studying the relative amplitudes of the teleseismic signals.

Attenuation of Fourier spectrum with distance

The parameters required to model the attenuation of strong ground motion are numerous because of its random nature. But the major contributing factors are the hypocentral distance to the station and the anelasticity and the scattering of the medium. The spectral amplitudes of the strong ground motion at a distance R can thus be expressed as[25]

$$\log_{10} A(f, R) = \log_{10} A_0 - \gamma(f) \log_{10} R + (\pi f \log_{10}(e)/Q\beta)R + S(f),$$

where A_0 is the amplitude of the source spectrum, R is the epicentral distance, γ is the co-efficient of geometric spreading, f is frequency, Q is the shear wave quality factor and $S(f)$ is the site response.

At short distances (up to 100 km), multiply reflected or refracted waves reaching the station are less, and it can be safely assumed that the attenuation is proportional to $R^{-\gamma}$, where γ is the coefficient of geometric

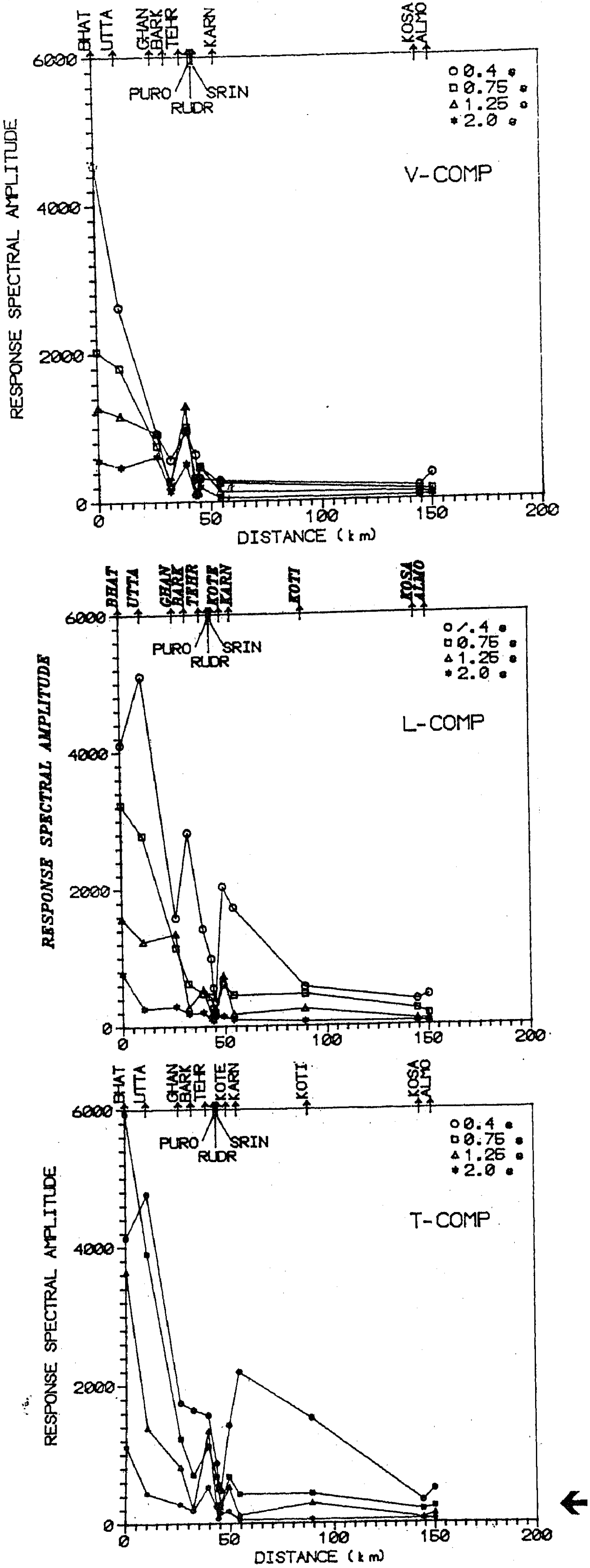

Figure 12. Decay of response spectra with distance for four periods.

spreading. The quality factor Q for the medium can be assumed to be constant, as for shallow earthquakes most of the wave energy is confined to the uppermost layer of the crust at short distances. A shear wave quality factor of 1000 is taken for this region based on the model given by Yu *et al.*[8].

The accelograms show that the shear phases are the most dominant in all the components and the P and its coda are weak. Hence the spectra from the whole accelerogram can be taken for the shear wave spectra. Using these assumptions, a regression is carried out for the coefficient of geometric spreading γ setting the site term to zero. The geometric spreading co-efficient is allowed to take different values for different distances and frequencies. The mean γ value thus obtained, need not be a true representative of the geometric spreading in the region, since it has accommodated the inadequacies in the assumption made for the regression. Using the value of γ in the above expression, the amplitude spectra are computed for all the stations. The differences between the observed and the computed spectra are taken as site responses. In Figure 13 the site responses thus obtained are shown for 5 stations in the distance range of 25 km from the source. Though the shape of the response matches with the response obtained from the spectral averaging method, the amplitude of the response in this method is larger by a factor of 2.5 or 3 in some cases.

The source spectrum and the site response function from the regression are used to normalize the observed spectral amplitudes to a common level in order to visualize the path effect. The variation of the normalized spectral amplitudes with distance for three different frequencies and for three components is shown in Figure 14. The data set is clustered in the distance range of up to 50 km. Only 2 points are available at about 150 km. Thus the results are better constrained for the shorter distance range. In this range the decay rates for all the three frequencies are the same within the resolution available. At larger distances there is a departure and the higher frequencies begin to show larger attenuation rates in a consistent manner.

Response spectrum and structural response records

A number of structural response recorders (SRRs) having natural periods 0.4 s, 0.75 s and 1.25 s are in operation throughout the country. At three locations, both the SRRs and the accelerographs have recorded the peak ground motions. A comparison of the readings at three periods 0.4 s, 0.75 s and 1.25 s for four stations is shown in Figure 15. While the values are comparable at

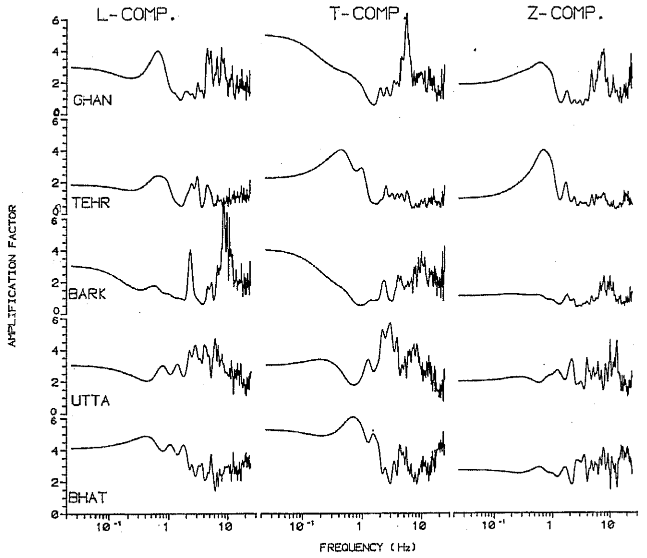

Figure 13. Site response functions obtained using regression technique (see text).

Bhatwari and Tehri, at the other two sites the SRRs values are considerably lower than the accelerograph values. At Uttarkashi, the SRRs values are proportionally lower at all periods. At Barkot, values are comparable at 0.4 s and 1.25 s while at 0.75 SRR give abnormally high value. This opens up the question of the fidelity of the SRRs.

Conclusions

The strong motion data for the Uttarkashi earthquake shows that the source spectrum for this earthquake is complex and may be represented by the superposition of two Brune spectra, one corresponding to the smooth slip over the whole fault plane and the other due to the roughness of fracturing over the fault plane corresponding to asperities and barriers. The corresponding stress parameters are 40 and 31 bars. The sites in the distance range of 40–50 km show significant amplification of response spectra. The sites at Tehri and Bhatwari show a considerable enrichment of low frequencies. Some sites

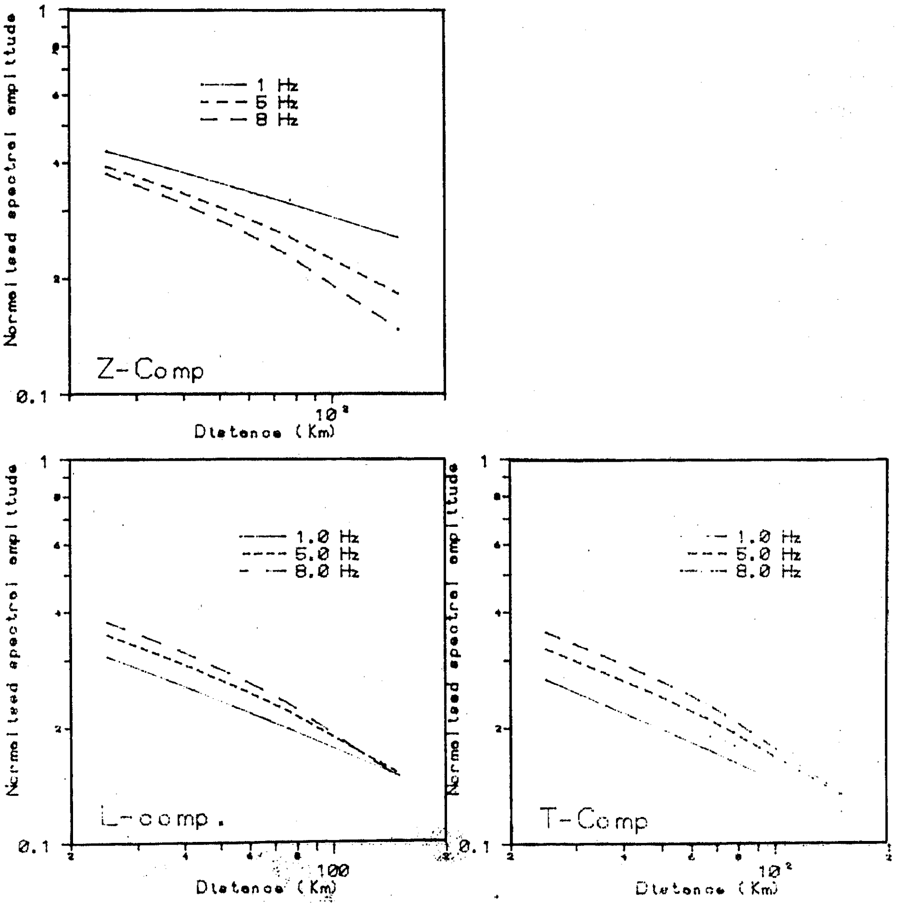

Figure 14. Dependence of the acceleration Fourier spectra on distance at three frequencies.

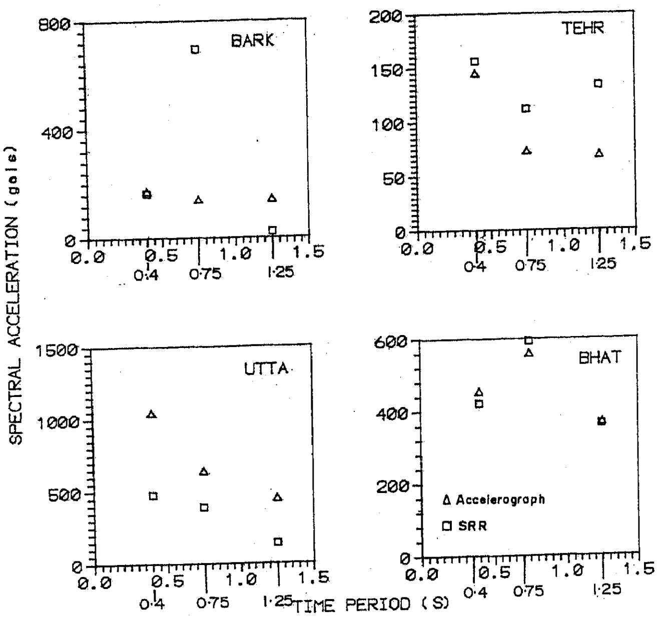

Figure 15. Comparison of the peak accelerations recorded using accelerographs and structural response recorders at the same sites.

show resonant narrow frequency bands towards the higher frequencies. The estimates of site amplification functions show amplifications in some frequency bands by factors of up to about 3. The amplification function at Tehri shows significant amplification by a factor of 2.5 in the period range of 1.0–2.5 s. The response spectra show site-specific characteristics. At Tehri there is a significantly larger response at longer periods (~1–2 s). These results show that assigning an average response spectrum for the purpose of earthquake-safe design of critical structures at such sites is not an adequate procedure. The attenuation of spectral ground acceleration with distance shows two distinct sections. For distances less than 60 km the attenuation is constant at all frequencies and for distances greater than 60 km the fall is more rapid for the higher frequency waves. The dependence of the peak ground acceleration on distance is better described by the relation based on the European data set[13] for the distances larger than 50 km while the relation based on the data set from Himalayan earthquakes[14] is better for the distance between 30 and 50 km.

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Development of insect-resistant transgenic plants using plant genes: Expression of cowpea trypsin inhibitor in transgenic tobacco plants

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Development of transgenic plants, engineered for insect resistance, has added a new dimension to crop improvement. The cowpea trypsin inhibitor (*CpTI*) gene offers resistance against a wide range of insects, and has been used for developing insect-resistant plants. In the present study, the *CpTI* gene, amplified by PCR, was cloned in a plant expression vector under the control of the CaMV 35S promoter and the NOS terminator, and introduced into model plant tobacco by *Agrobacterium*-mediated transformation. The presence and expression of the *CpTI* gene in primary transformants R_0 and R_1 progeny was confirmed by PCR and immunobiochemical analyses. The efficacy of the expressed CpTI protein against *Spodoptera litura* was tested by feeding trial larvae under laboratory conditions. Reduction to the extent of 50% was observed in the biomass of *S. litura* larvae fed on transgenic leaves, expressing 3–5 μg CpTI/g fresh tissue.

PLANTS have several defence mechanisms against insects and other pathogens, involving defence genes whose products are detrimental to such invasions. One of these defence mechanisms involves proteinase inhibitors, which are insecticidal in nature. Serine proteinase inhibitors such as soybean trypsin inhibitor, cowpea trypsin inhibitor (*CpTI*), potato/tomato trypsin inhibitor (*PTI*) I and II, being antimetabolic agents, have been shown to be toxic against lepidopteran insects[1–3] and interfere in their growth and development.

The developments in genetic engineering techniques provide a means of transferring such insecticidal genes from one organism to another, including agronomically important crops, for better yield through reduced crop losses. Unlike classical breeding, this technique avoids cotransfer of undesirable genes by being selective for the target gene. Moreover, it does not pose any threat to the environment unlike many other chemical pesticide sprays. The crystal protein gene from *Bacillus thuringiensis* (*Bt*) has already been introduced in many plants such as tobacco[4], tomato[5], potato[6], cotton[7] and maize[8] and has been shown to provide protection against insect pests. However *Bt* genes, as such, may not express as efficiently in plant systems and require extensive codon modifications to suit the plant genome[9,10]. In contrast, the advantage of using plant insecticidal genes over bacterial genes is that they can be introduced to other plant systems without much modification. It has earlier been shown that the transfer of proteinase inhibitor (a trypsin inhibitor) gene from cowpea[11], potato[12,13] and also one amylase inhibitor gene[14] to tobacco or other test plants greatly reduced the damage caused by the insect pests.

In this paper, we present data on the development of transgenic tobacco plants through transfer of *CpTI* gene and demonstrate that the transgenic plants restrict the growth of *Spodoptera litura*. The *CpTI* gene has already been tested by other workers to be used as insecticidal gene. The gene is small (240 bp) and the absence of introns makes it easier to handle. Secondly, since the *CpTI* acts by binding the catalytic site of trypsin, resistance developed by insects against *CpTI* through mutations in the catalytic site of trypsin would be minimal[11]. Also, it has been reported earlier that the gene is effective against a variety of insects such as *Spodoptera litura*, *Heliothis virescens* and *Manduca sexta*. The study suggests potential for optimization of the expression of *CpTI* to confer resistance against insect pests. To the best of our knowledge this is the first report of successful introduction of indigenously isolated and characterized *CpTI* gene into transgenic tobacco. The preliminary work on CpTI protein purification, antibody production, gene isolation and sequencing has already been reported[15].

Standard techniques of molecular biology as described by Sambrook *et al.*[16] were used for the construction of plasmids. The *CpTI* gene, earlier cloned by us in pBluescriptII KS(+) was excised using *Bam*HI and *Sac*I enzymes and was subcloned in the plant expression vector pBI121 at the same sites after removal of the β-glucuronidase (*GUS*) gene from the vector. The construct was named as pBTI and was under the control of single CaMV 35S promoter and NOS terminator.

The resultant binary plasmid pBTI was then mobilized into *A. tumefaciens* LBA 4404 by triparental mating. For this, *E. coli* cells containing pBTI, LBA4404 cells and helper cells (containing pRK2013) were grown overnight in the LB medium containing kanamycin, rifampicin–streptomycin and streptomycin–kanamycin respectively. Cells were harvested and suspended separately in fresh LB medium (minus drug). 5 μl of each cell suspension was plated one over the other on a LA plate containing all the three drugs and allowed to grow for 2 days at 28°C. Single colonies of transformed *Agrobacterium* were analysed for the *CpTI* gene and a positive clone pBTIA was used for tobacco infection.

Transgenic plants were developed from tobacco leaf discs (*Nicotiana tabacum*, cv. *Petit havana*) by cocultivation method[17]. Leaves were surface-sterilized and 5 mm discs were cocultivated for 2 h with pBTIA in MS medium with gentle shaking. Regeneration medium for shooting consisted of MS supplemented with 0.2 mg/l

1-napthaleneacetic acid (NAA) and 1 mg/l 6-benzylaminopurine (BAP), 100 µg/ml kanamycin and 500 µg/ml cefotaxime. After eight weeks, shoots were rooted in phytohormone-free MS medium containing 100 µg/ml kanamycin. After aqua-hardening, the regenerated plants were transferred to pots, grown in the glasshouse and self-pollinated. Mature seeds were collected and tested for germination in the absence or presence of kanamycin (150 µg/ml). Kanamycin-resistant plants were used for progeny analysis.

Total genomic DNA was isolated from R_0 and R_1 tobacco leaves according to the method described by Kochko and Hamon[18]. Tobacco leaves (1 g) were ground in liquid N_2 and homogenized in 10 volumes of extraction buffer (50 mM tris HCl pH 8.0, 50 mM NaCl, 5 mM EDTA, 10 mM BME). 1.25% SDS was added to the homogenate and it was incubated at 65°C for 10 min. After the addition of 1.25% potassium acetate, the mixture was incubated in ice for 20 min and centrifuged at 13000 *g*. Precipitation of DNA was carried out by the addition of 0.5 volumes of isopropanol to the filtered supernatant and incubation at –20°C for 30 min. The nucleic acid pellet obtained after centrifugation was subjected to RNAase treatment and phenol–chloroform extraction. DNA was collected from the aqueous phase by isopropanol (0.7 vol) precipitation.

DNA, thus obtained, was tested for the presence of *CpTI* gene by PCR amplification using the CpTI-specific primers[15].

Tobacco leaf extracts were prepared according to Johnson *et al.*[12] at 4°C. Leaf tissue (0.5 g) was homogenized in liquid nitrogen in an isolation buffer containing 0.1 M tris (pH 7.0), 0.5 M sucrose, 0.1% cysteine and 50 mM BME. The homogenate was centrifuged at 10000 *g* for 10 min and the supernatant was saturated to 50% with ammonium sulphate. The precipitate was collected by centrifuging at 2500 *g* for 10 min and it was suspended in 50 mM ammonium carbonate, lyophilized and resuspended in water. The brown colour of the suspension was removed by adding small amount of resin (mixed bed resin, Sigma). After centrifugation, protein was concentrated in a Speed Vac (Savant) at room temperature. This extract was used for the immuno-biochemical analyses. Proteins in the extract were estimated by Peterson's method[19].

Proteins from the leaf extracts of the transformed and untransformed plants were separated on 15% SDS-PAGE[20]. Protein samples were prepared in 60 mM tris HCl pH 6.8 containing 0.1% SDS, 20 mM DTT and 0.125% bromophenol blue. Samples were boiled for 1 min before electrophoresis. After electrophoresis, the proteins in the gel were transferred on a PVDF membrane (Bio-Rad). The protein blots were first blocked with 0.5% casein, treated with anti-CpTI IgG[15] followed by goat anti-rabbit IgG alkaline phosphatase conjugate. The blots were developed with BCIP (0.06 mg/ml) and NBT (0.1 mg/ml) in a buffer containing 0.1 M tris HCl pH 9.6, 0.1 M NaCl and 5 mM $MgCl_2$.

The trypsin inhibitor activity was assayed by the colorimetric method[21]. Each reaction was carried out for 15 min at room temperature (25–26°C) using 1 µg of trypsin and α-*N*-benzoyl-L-arginine-*p*-nitro anilide (BAPNA) as substrate. The amount of nitroaniline released by trypsin with and without leaf extract in control and transgenic plants was measured spectrophotometrically (Kontron) at 410 nm. Assays were carried out using fresh leaf extracts.

Four-day-old larvae (0.5 mg/insect) of *Spodoptera litura* were used for feeding trials. The experiments were run in triplicates. For each set, 10 insects were placed in a beaker and fed on fresh and thoroughly washed tobacco leaves. Beakers were covered with markeen cloth to avoid escape of insects. Insects were weighed on alternate days for 10 days, changing leaves every time. Insecticide sprays were avoided for atleast one month before feeding trials.

Tobacco leaves were transformed with transformation plasmids, pBI121 and pBTI. The pBI121 is an expression vector containing *GUS* gene whereas in pBTI, the *GUS* gene was replaced by the *CpTI* gene fragment (Figure 1). Plants transformed with pBI121 were used as

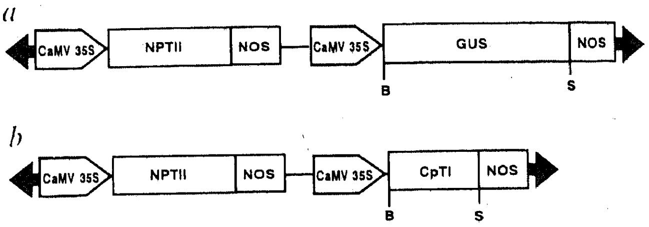

Figure 1. Structure of binary plasmid constructs containing the *CpTI* gene. *a*, Binary plasmid vector pBI121; *b*, Binary plasmid pBTI in which the GUS fragment was replaced by *CpTI* gene at *Bam*HI and *Sac*I sites. B, *Bam*HI; S, *Sac*I; NOS, nopaline synthase polyadenylation region; CaMV 35S, Cauliflower mosaic virus 35S promoter; GUS, *β* glucuronidase gene; NPTII, Neomycin phosphotransferase gene.

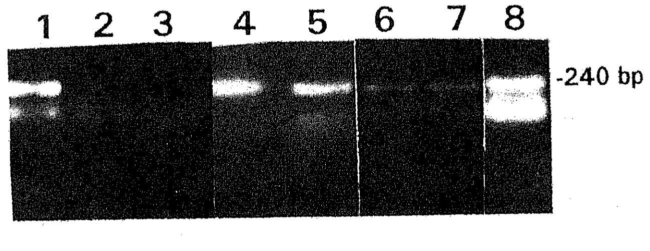

Figure 2. PCR analysis for the detection of *CpTI* gene in transgenic tobacco plants. Plasmid pTIVO containing *CpTI* gene (lane 1), total DNA from untransformed plant (lanes 2), pBI121 transformed plant (lane 3), primary transformants V_1, V_4 (lanes 4, 5), R_1 generation plants V_1 – 10, V_1 – 12, V_4 – 10 (lane 6–8 respectively) were analysed using *CpTI* gene specific primers. PCR products were separated on 1.2% agarose gel, stained with ethidium bromide and photographed under ultraviolet light.

a negative control. Twenty-five independent pBTI transformed plants were obtained on the basis of the kanamycin resistance (neomycin phosphotransferase II gene). Ten of these plants were randomly picked and tested for the presence of the *CpTI* gene. Using primers specific for the *CpTI* gene, all the ten plants showed 240 bp *CpTI*-specific fragment after electrophoresis of PCR amplified products. As expected no band was observed in untransformed or in pBI121 transformed plants (Figure 2). R_1 seeds of some of R_0 plants, viz. V_1, V_2, V_4, V_5, V_{20} etc., which were obtained by selfing, were germinated in the presence of 150 µg/ml kanamycin. Seedlings were segregated in the ratio of 3:1 when selected for kanamycin (Table 1). Seedlings of untransformed tobacco seeds turned yellow in kanamycin. R_1 progeny of V_1 and V_4 plants also inherited the *CpTI* gene as indicated by PCR analysis of DNAs of these plants which showed the 240 bp band (Figure 2). Progeny of other R_0 plants was also analysed (data not shown) to confirm the presence of *CpTI* gene.

Ammonium sulphate-precipitated proteins from transformed and non-transformed tobacco leaves were electrophoresed on a 15% SDS-PAGE and Western blots were probed with anti-CpTI IgG[15]. Immunoanalysis (Figure 3) showed a single band of 16.5 kD corresponding to purified CpTI protein in $V_1 - 10$, $V_1 - 12$, $V_1 - 8$ plants (V_1 progeny, lanes 1, 2, 3) and $V_4 - 10$ plant (V_4 progeny, lane 4). This band was absent in the extracts of untransformed plant CR_1 (lane 5) as well in plants transformed with pBI121 alone (not shown). Although the CpTI is a 8.0 kD protein in monomeric form, it exists in dimeric/multimeric forms (a characteristic of Bowman–Birk inhibitors) even under reducing conditions[22]. Purified CpTI protein from cowpea also shows a molecular weight of 16.5 kD on SDS-PAGE[15] in 50 mM BME. The above results indicate that *CpTI*, when expressed even in tobacco, retains its characteristic dimeric form.

Table 1. R_1 inheritance of the *NPTII* gene

R_0 transgenic lane	KanR	KanS	χ^2 value ($P = 0.05$)
V_1	68	20	0.24
V_2	78	22	0.48
V_4	48	12	0.80

R_0 seeds were grown in the presence of 150 µg/ml kanamycin. KanR indicates R_1 plants carrying *NPTII* gene and KanS indicates plants without the *NPTII* gene.

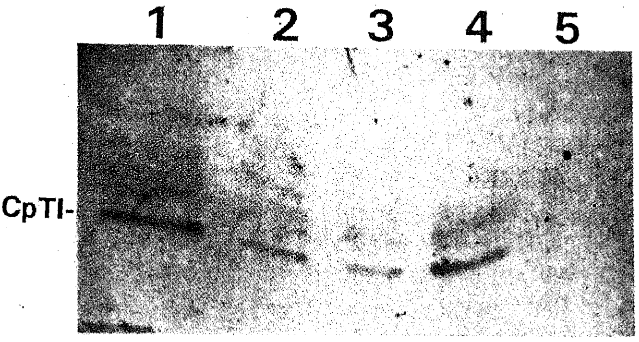

Figure 3. Western blot of CpTI protein from tobacco plants transformed with the pBTI construct. Total proteins were isolated and separated on a 15% SDS-PAGE as described in text. Lanes 1–4, protein extracts from various R_1 generation plants, viz. $V_1 - 10$, $V_1 - 12$, $V_1 - 8$ and $V_4 - 10$ respectively; lane 5, protein extract from untransformed tobacco plant.

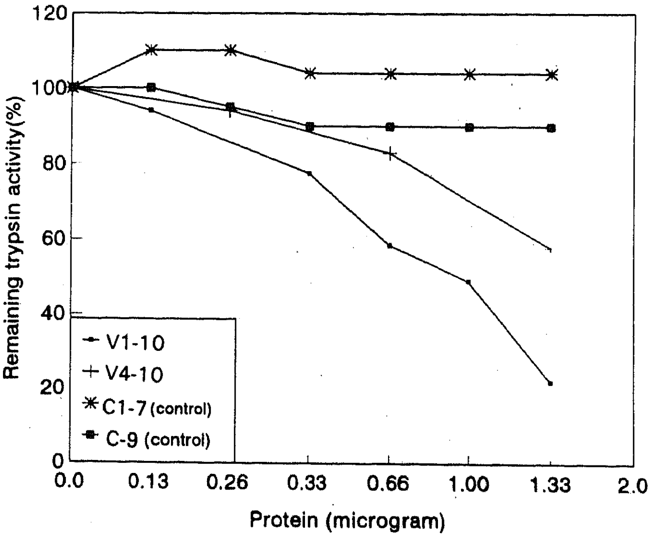

Figure 4. Inhibition of trypsin by tobacco leaf extracts from transgenic plants. 50% ammonium sulphate precipitated extracts were used for inhibitor studies. Activity was assayed as described in text. $V_1 - 10$, $V_4 - 10 = R_1$ generation plants expressing CpTI protein; $C_1 - 7 = R_1$ progeny of pBI121 transformed tobacco plant; C–9 = untransformed plant.

Trypsin inhibitor activity was measured as per cent decrease in trypsin activity. We tested R_1 progeny of the various R_0 plants and it was found that the progeny of V_1 plants showed the highest inhibition of trypsin activity. Figure 4 shows that leaf extract from the control plants did not inhibit trypsin activity whereas leaf extracts from $V_1 - 10$ (V_1 progeny) and $V_4 - 10$ (V_4 progeny) inhibit the trypsin activity. The V_1 progeny by itself showed variations in *CpTI* inhibition. This was also indicated by different levels of *CpTI* accumulated in these plants. For example, $V_1 - 10$ leaves accumulated 5 µg CpTI/g fresh tissue which is higher than the CpTI protein accumulated in V_1– 12 plant (3 µg/g fresh leaf). This expression is quite low when compared with the value of 50–200 µg/g tissue for other proteinase inhibitors in transformed tobacco plants driven by CaMV 35S promoter[12].

The R_1 progeny of V_1 and V_4 plants, which showed the presence of *CpTI* gene by PCR and Western blots, were used for insect bioassays. These experiments were repeated thrice and each experiment was run in triplicates. The results of these experiments are shown in Figure 5. The overall growth was reduced when insects were fed on positive plants (containing *CpTI*) in comparison to untransformed plants or plants transformed with pBI121. R_1 progeny of V_1 plants showed higher resistance than the progeny of other R_0 plants. There were variations in growth reduction and mortality in different experiments but V_1-10, V_1-12 and V_2-4 consistently showed better resistance. There was more than 50% reduction in biomass of the insect larvae fed on these plants compared to controls. The insects also showed a refractory tendency (i.e. going away from the leaf). Data for a typical feeding experiment is shown in Figure 6 in which a continuous change in insect biomass is shown when larvae were fed on CpTI-transformed and control tobacco leaves. The data showed that at the end of 10 days V_1-10 and V_1-12 fed larvae had only 40% and 60% weight as compared to larvae fed on leaves of control tobacco plants (Table 2). Figure 7 shows the reduction in the growth of larvae fed with the leaves of transgenic plants, while Figure 8 shows the extent of leaf damage in control and transgenic (CpTI) plants by the *S. litura* larvae during the same period. These data clearly indicate that the introduction of the *CpTI* gene in tobacco plants results in substantial reduction in the growth of insect larvae. The stability of the introduced gene was evident in the R_2 progeny (data not shown) and inheritance in the following generations is being tested.

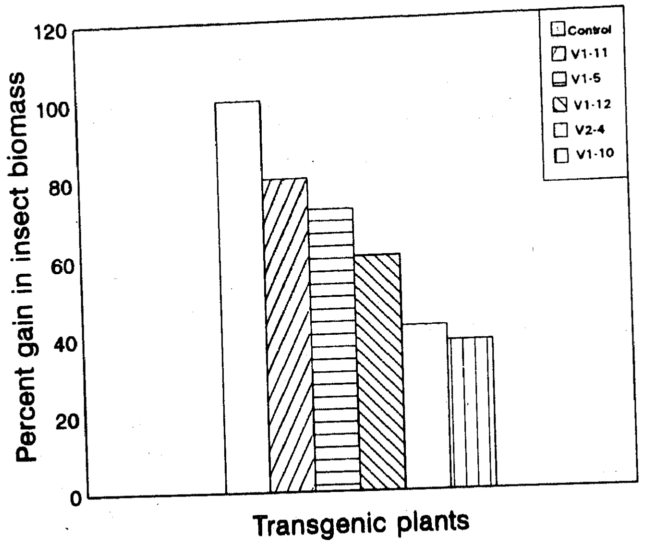

Figure 5. Bioassay data for 10-day feeding trials of *Spodoptera litura* larvae on transgenic tobacco plants. Different bars show per cent gain in larval weight when fed on transgenic and control tobacco leaves.

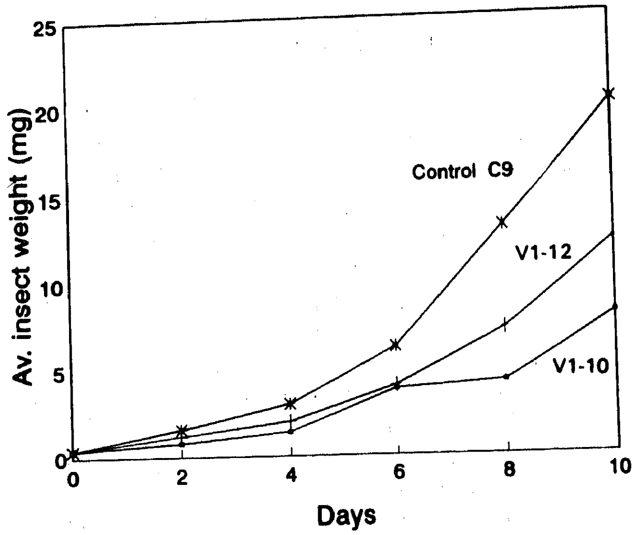

Figure 6. Continuous growth pattern of *Spodoptera litura* on a diet of transgenic tobacco leaves. Larvae were fed on leaves from untransformed plant C-9 (*–*) or leaves from V_1-10 (●–●) and V_1-12 (x–x) transgenic plants expressing CpTI protein. The insect weight shown represents the average weight per insect.

Table 2. Bioassay of R_1 transgenic tobacco plants against *S. litura* larvae

Plant	Initial no. of insects	Initial wt per insect (mg)	% mortality after 10 days	Final wt per insect (mg)	% wt of control
C-9	10	6 ± 0.7	0	20.8 ± 2.8	100
C-7	10	5 ± 1	0	20.0 ± 1.6	96
V_1-10	10	4 ± 0.7	40	8.3 ± 0.5	40
V_1-12	10	5 ± 0.5	37	12.3 ± 2.3	59
V_2-4	10	4 ± 1	35	8.5 ± 3.6	41

Selfed progenies of R_0 transformants were used for bioassays. Assays were carried out in triplicates. See text for details. Data shown is average of 3 assays.

In the present study, tobacco transgenic plants were developed carrying *CpTI* as a transgene. We had earlier reported cloning of the trypsin inhibitor gene from cowpea, its expression in *E. coli* and identification of expressed protein by Western blot analysis[15]. The expression of the *CpTI* gene in the present study was driven by a single CAMV 35S promoter in the construct used for plant transformation. The presence of the gene in the regenerated plants was confirmed by PCR and Southern analysis (data not shown) whereas its expression was tested by immuno-biochemical analyses. The toxicity trials (bioassays) were conducted on the larvae of *Spodoptera litura*, which clearly indicated that the expressed protein was physiologically active and provided reasonable resistance. While the transgenic plants were found to be resistant to insect larvae, problems with regard to low accumulation of CpTI protein was encountered. It was, for example, observed that even the most toxic plants accumulated only 3–5 μg CpTI/g tissue or approximately 0.05% of soluble protein fraction. Hilder *et al.*[11] reported high CpTI expression in their transgenic tobacco plants (1% of total soluble protein).

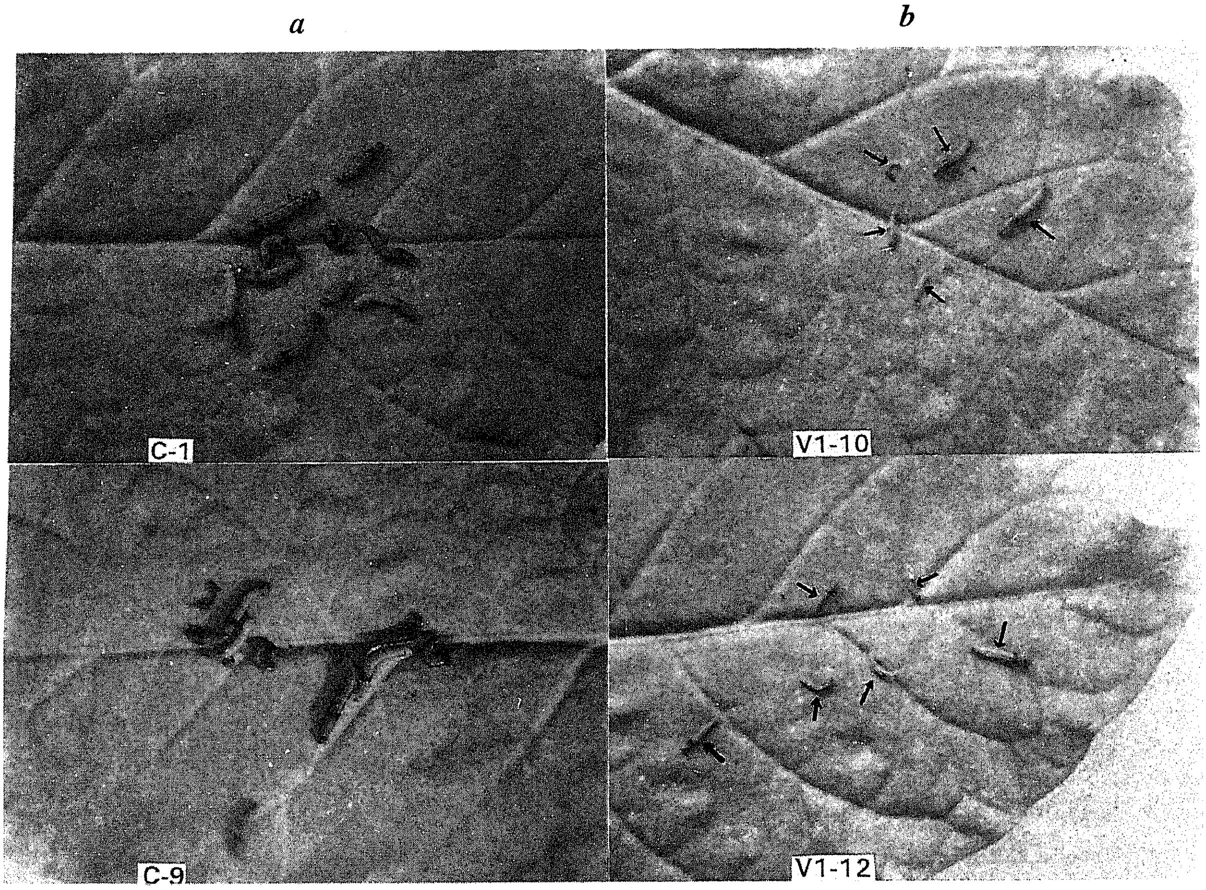

Figure 7. Growth reduction of *S. litura* larvae fed on transgenic plants expressing *CpTI* gene after 10-day feeding trial. *a*, Larvae grown on control plants C–1 and C–9; *b*, Larvae grown on transgenic plants $V_1 - 12$ and $V_1 - 10$.

Johnson *et al.*[12] have also reported higher levels of tomato and potato proteinase inhibitors in their primary transformants. However, it was interesting to observe that even with this low expression, our transgenic plants were able to show resistance to the larvae of *S. litura.* Further modifications in the construct are being done to increase the expression of *CpTI* and higher accumulation of the protein. Several reasons could be attributed for the low expression of the transgene. We have used only coding sequences of the *CpTI* whereas Hilder *et al.*[11] have added 200 bp upstream region of the gene which might be providing stability to the mRNA. Study of Masoud *et al.*[23] on transgenic tobacco plants obtained by transferring 14 K-Cl gene (encoding corn bifunctional inhibitor) also reported low protein accumulation (5–6 µg/g tissue). They suggested post-transcriptional events as the major factor for the low accumulation. Methylation and gene silencing are the other phenomena which may result in the lower expression of transgenes[24–26]. The gene silencing phenomenon is more pronounced when multiple copies of the foreign gene are integrated into the genome. However, in our studies, the *CpTI* gene was found to be integrated only in single copy (Southern analysis, data not shown). However, the possibility of gene silencing can still not be ruled out totally. We have not tested the possibility of *CpTI* gene or its promoter getting methylated. The variability in resistance shown by plants from R_0 to R_1 generation was reported earlier by others in their bioassays[11]. However, it was attributed to inherently variable biological systems rather than variation in the expression.

Our main aim was to develop an indigenous system to stably transform crop plants with *CpTI* gene, as ready-made constructs of *CpTI* were not available. We have shown successful transformation of tobacco plant with the *CpTI* gene, which will pave way to move this gene into other crop plants. Although some data on the expression of this gene in plants show that it is not as effective as some of the *Bt* genes, its utility as a supplementary gene to enhance the effect of other insecticidal genes cannot be overlooked. Such an approach is desirable to restrict development of resistance by insect pests toward products of transgenes. The multigene protection strategy seems to be an attractive

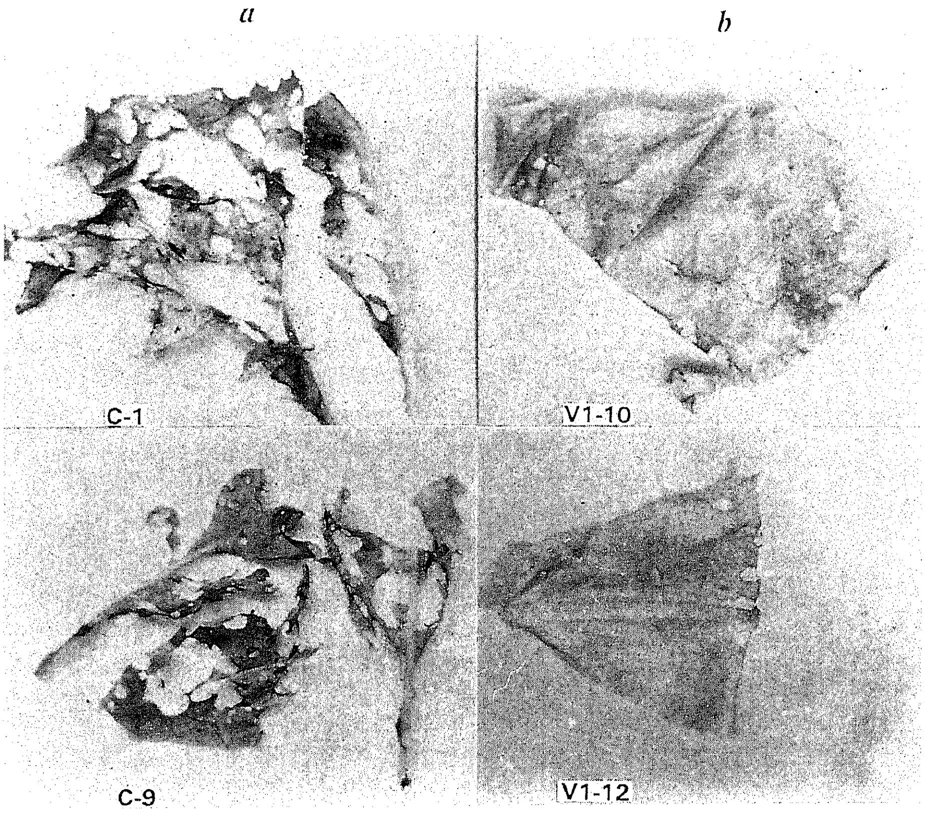

Figure 8. Leaf damage of *Spodoptera litura* fed on leaves from transgenic plants expressing *CpTI* gene. *a*, C-1 and C-9 control plants; *b*, V_1 – 10 and V_1 – 12 transgenic plants.

possibility and protease inhibitor, amylase inhibitor[27,28], lectin[29], etc. could provide genes of choice for this purpose.

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Reproductive success of large and small flies in *Drosophila bipectinata* complex

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Reproductive success of large and small flies of *Drosophila malerkotliana* and *D. bipectinata* has been studied using no-choice method. Results indicate that large males have higher remating ability and longevity than small males as a result of which they can inseminate more females in their lifetime than small males. Large females also have higher reproductive success because they have more number of overioles, lay more eggs and produce more fertile offspring than the small female and also mate with more males in their lifetime than small female by having higher longevity. These findings suggest that large flies have higher reproductive success than small flies.

BODY size is the most obvious, easily observable and measurable phenotypic trait and there is compelling evidence indicating that it is directly related to fitness[1]. In *Drosophila*, as in many organisms, body size is closely linked to life history traits such as fecundity, dispersal ability and mating success[2–4] and has been widely used in studies on quantitative genetics[5]. The adaptive nature of body size in *Drosophila* has also been demonstrated by many workers both in natural populations[6,7] and in laboratory populations[8,9]. Body size also influences mating speed, fecundity and other fitness characters[10–13].

Many workers have used thorax length as an index of body size in *Drosophila*[12–14]. Apart from thorax length, other morphological traits such as wing length, wing width and face width have been used as index of body size. Wing is another phenotypic trait which can be used as an index of body size[15,16]. Relationship between wing length and mating speed had been studied in *D. malerkotliana*[16]. Correlation between copulation duration and fertility; and variation in mating propensity had also been studied in *D. bipectinata*[17–19]. The fitness characters such as fecundity, fertility, male remating ability and longevity of large and small flies have not been made. Moreover, mating speed alone does not qualify an individual to have higher reproductive success. Hence the present investigation is aimed at understanding reproductive success of large and small flies in *D. bipectinata* species complex.

The stocks used in the present study were *Drosophila malerkotliana* and *D. bipectinata*. All experiments were made separately for each of these two species. The stocks used in the present study originated from 150 naturally inseminated females from Mysore, Karnataka. When progeny appeared, flies were distributed to different culture bottles and were maintained under constant temperature (22 ± 1°C). For every generation, flies multiplied in different culture bottles were mixed together and eggs were collected using Delcour's[20] procedure. Eggs (100) were seeded in fresh quarter pint milk bottles with 25 ml of wheat cream–agar medium to avoid larval competition during development (this procedure allows us to reduce environmental variation in size). After 10 generations, when adults emerged, virgin females and males were isolated within 3 h of their eclosion and maintained separately at 22 ± 1°C. Wing lengths of male and female flies were measured separately when they reached the required age. Each fly was etherized individually, the intact left wing kept in horizontal plane was measured from humeral cross vein to the tip with an ocular micrometer at 100 × magnification. Wing length was measured in units of 1/10 mm. After measuring the wing length, each fly was placed separately in fresh food vials to study fitness characters.

To study fecundity and fertility, five to six-day-old virgin females and bachelor males of chosen wing size (see Table 1 for chosen wing size) were taken and different crosses were made (large male × large female; large male × small female; small male × large female; small male × small female). Soon after mating, mated females were transferred into fresh food vials every 24 h without etherization. The total number of eggs laid in each vial and the total number of progeny appeared were counted over a period of 15 days. 50 trials were run for each cross and mean fecundity and fertility were obtained.

To study the remating ability of male, 5 to 6-day-old virgin females and bachelor males of chosen wing size (see Table 1 for chosen wings size) were taken and different crosses were made (large male × large female; large male × small female; small male × large female; small male × small female). When mating occurred, the pair was allowed to complete copulation. After

Table 1. Fitness characters of different crosses

Parameters	Large male × Large female	Large male × Small female	Small male × Large female	Small male × Small female	*F*-value
A: *Drosophila malerkotliana*					
	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	
Fecundity	313.24 ± 4.01	281.35 ± 6.07	309.27 ± 5.57	268.09 ± 0.58	15.84*
Fertility	305.71 ± 3.87	268.86 ± 5.02	303.00 ± 5.04	233.86 ± 5.55	43.27**
Remating ability of male	5.66 ± 0.14	5.56 ± 0.13	3.00 ± 0.08	3.46 ± 0.14	28.47**
B: *Drosophila bipectinata*					
Fecundity	262.39 ± 2.91	215.40 ± 2.36	249.38 ± 3.09	199.34 ± 1.81	133.17**
Fertility	249.79 ± 2.54	204.00 ± 2.87	236.11 ± 2.94	186.84 ± 2.13	125.56**
Remating ability of male	4.84 ± 0.17	5.52 ± 0.25	3.20 ± 0.18	3.64 ± 0.21	16.05*

Mean values are reported with standard error $*P < 0.05$; $** P < 0.01$.

	D. malerkotliana	*D. bipectinata*
Large male	(17.06 units)	(17.28 units)
Small male	(14.34 units)	(14.04 units)
Large female	(18.39 units)	(19.36 units)
Small female	(15.88 units)	(16.32 units)

Wing length of large and small flies is given in parenthesis.

copulation, the mated female was aspirated out and replaced with another virgin female and the same procedure repeated. Observation was made for 2 h and the number of females mated by single male was recorded. If there was no mating within 2 h, then the pairs were discarded. The remating ability of 50 males (trials) were studied for each cross and mean remating ability of male was calculated.

One-way analysis of variance was carried out on mean values of fecundity, fertility and remating ability of male.

Five to six days old large and small females of chosen wing length (see Table 1 for chosen wings size) were used to determine the number of ovarioles in them. Individual females were separately dissected out in a drop of physiological saline under binocular stereomicroscope. The ovarioles of each ovary were separated from each other with a fine needle and counted. Student's *t* test was carried out on mean number of ovarioles/individual of 25 large and 25 small individuals.

To measure longevity, virgin females and bachelor males of chosen wings size (see Table 1 for chosen wings size) were taken and different crosses were made as above. One female and a male were transferred into fresh food vials containing wheat cream–agar medium. Active yeast was also added to vials 48 h prior to use. Vials were renewed daily during measurement of longevity. All cultures were maintained at 22 ± 1°C and at a 12:12 light/dark cycle. Adult longevity is measured here from the date of completed eclosion to the death of an individual. A total of 50 replicates was made for each cross.

Sexual selection is common among animals because the female's reproductive success is limited by the number of eggs she can produce in her lifetime and a male's reproductive success is limited by the number of females he can inseminate. In the present study, data on fecundity and fertility (Table 1) show the advantage of large size in both *D. malerkotliana* and *D. bipectinata*. Both fecundity and fertility were highest in crosses involving males and females with long wings and lowest in crosses involving females with short wings. This is in conformity with the work of Singh and Mathew[21,22] who, while working in *D. ananassae*, reported that flies possessing high number of sternopleural bristles are larger in size than those with low number of bristles. The flies with high number of bristles show greater mating success and produce more progeny than those with low number of bristles.

Table 1 also shows the data on remating ability of male in different crosses. According to these data, the remating ability of large male crossed with large female was more when compared to small male crossed with small female, indicating that a large male can inseminate more females and produce more fertile offsprings than a small male. The data presented in Table 1 also show that males of both *D. malerkotliana* and *D. bipectinata* have remating ability. In *Drosophila*, males of many species have this ability[23–25]. Table 1 also show that a large male has higher remating ability than small males. The remating ability was highest when the large male was allowed to mate with large female. With higher remating ability and aggressiveness, the large male can inseminate more females in a multiple-choice situation or in nature and increase its fitness than small males. Even in *D. bipectinata*, though the remating ability was slightly different from that of *D. malerkotliana*, the larger male had higher remating ability.

The size of the ejaculate is also an important factor which determines the fitness of males. No direct evidence could be obtained on the size of the ejaculate or the number of sperms that enter the female genital tract. In *Drosophila* when copulation occurs, the sperms are stored in the spermatheca and fertilized eggs are released. The female is able to receive the second male only when most of the sperm in the spermatheca are exhausted. Thus the female remating interval provides some evidence on the size of the ejaculate. Our unpublished data on remating interval show that when a large female is mated by a small male, the remating interval is short (in terms of days) because it receives lesser number of sperms and it has to receive the males more frequently than the large male. When a small female is mated by a large male, the remating interval is long and confirms that a large male has an advantage over small males. According to Gruwez *et al.*,[26] higher fecundity of female is correlated with number of ovarioles present in it. The present data on wing length and number of ovarioles (Figure 1) show that large females have more number of ovarioles than small ones. These observations agree with the findings of Montague[27] who demonstrated that larger females carry more ovarioles and have higher potential fecundity and Santos *et al.*[13] who found positive correlation between thorax length and number of ovarioles in *D. buzzatii*.

Large males are capable of inseminating more females than small males[11] and transfer more sperm per ejaculate. Large females with more number of ovarioles when mated by a large male therefore can receive and store more sperms than small females and this offers greater fitness advantage for large individuals.

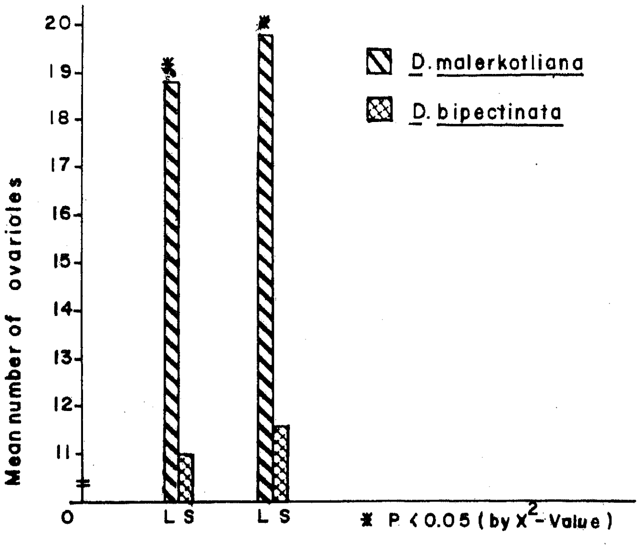

Figure 1. Mean number of ovarioles of large and small females, L, large female; S, small female.

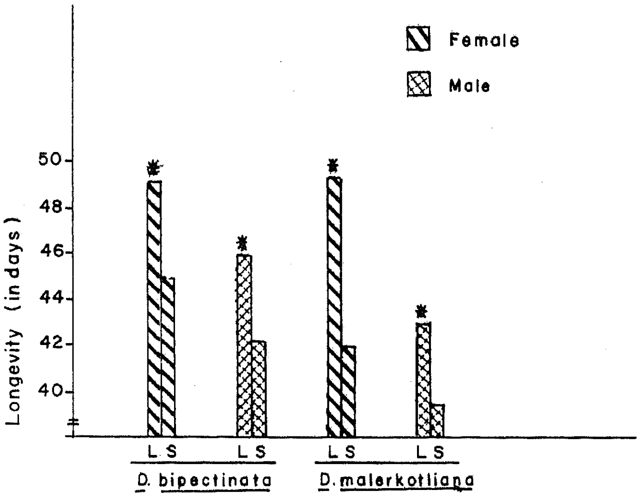

Figure 2. Longevity of large and small flies. L, large, S, small; *, $P < 0.05$ (by χ^2-value).

Higher reproductive success of large flies can also be accounted from its longevity. From Figure 2 it is clear that large flies have higher longevity than small flies in both *D. malerkotliana* and *D. bipectinata*. Partridge and Farquhar[11] have pointed out that larger males have been demonstrated to have greater longevity. In contrast to this we found both large male and female have greater longevity than small male and female. Large males with higher longevity could inseminate more females than small males and as a result the total number of progeny they produce is greater. Higher longevity of large females could also give an opportunity to mate with more number of males in their lifetime and produce more offspring than small female. Thus it is evident that the large males have higher reproductive fitness because they are able to mate more females by their higher remating ability compared to small males and they also have higher longevity than smaller males as a result of which they can inseminate more females in their lifetime than small males. Large females also have higher reproductive success because they have more number of ovarioles, lay more eggs and produce more fertile offsprings than the small female and also mate with more males in their lifetime than small female by having higher longevity.

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Deformational features in the river bluffs at Ter, Osmanabad district, Maharashtra: Evidence for an ancient earthquake

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Deformational features developed on the sedimentary sections at Ter, Osmanabad district in Maharashtra offer evidence for a spatially restrictive, but a damaging earthquake in the vicinity. The seismogenic features exposed in the sedimentary sections include flexures, warps, buckle folds and vertical offsets. Apparent trend of these structures suggests their formation under regional compressive stresses acting in the northeast-southwest direction. The radiocarbon age data obtained on the carbonaceous materials from the section indicate that the causative processes must have occurred ~1500 years ago. These structures, located 40 km northwest of Killari, the site of 1993 earthquake, add a new dimension to the question of spatio-temporal characteristics of earthquake generation in the Deccan Trap region.

OBTAINING evidence for past earthquakes is an important part in the seismic hazard evaluation of a region. This is particularly relevant in the shield regions where the historical seismicity data are meagre. And, most often these earthquakes recur at intervals longer than the recorded human history. Regions where large earthquakes have occurred in the recent years provide type areas to look for relics of past activity. Accordingly, we investigated several sites around Killari for possible indications of previous deformation[1-4]. One locale of interest was Ter which lies on the west bank of the river Terna, and is located 40 km, northwest of Killari and 18 km to the north-northeast of Osmanabad (Figures 1 *a* and *b*).

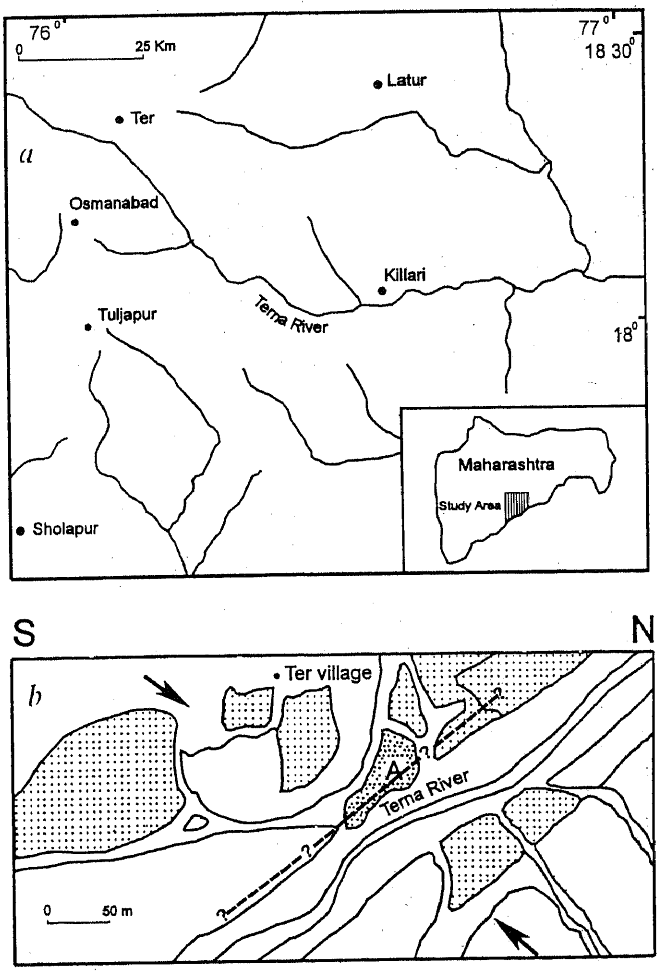

Figure 1 *a*, Location map of the area; *b*, Location of river bluffs at Ter. The section under study is denoted by 'A'. Arrows indicate direction of σ_1. Dashed line indicates probable trend of the seismogenic fault.

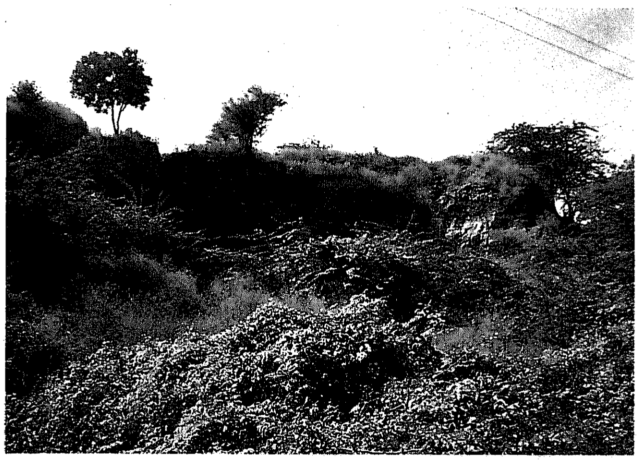

Figure 2. Photograph showing the sedimentary section ('A' in Figure 1 *b*) at Ter.

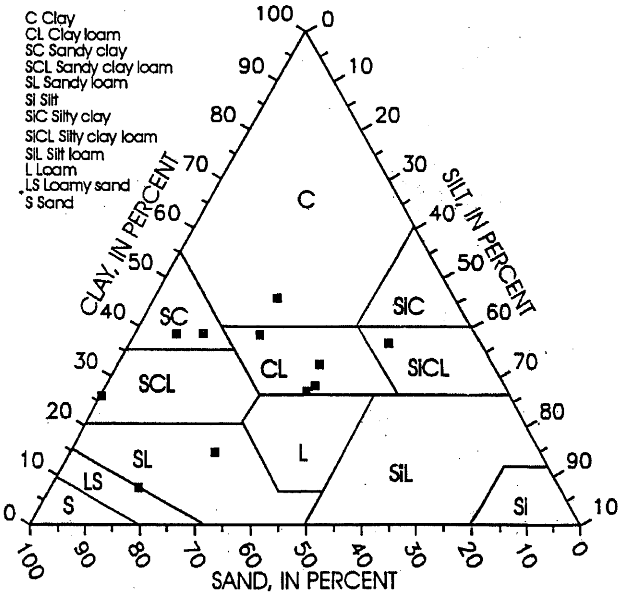

Figure 3. Grain size distribution of Ter sediments, based on the US Department of Agriculture textural classes (Soil Survey Staff)[22].

We examined these ~10-m-high sections (Figure 2), in order to find out the geological evidences of previous earthquakes. This paper presents the deformational structures deciphered from the river bluffs at Ter, their causative factors and implications.

Several bluffs occur along the western bank of the river Terna near village Ter. The bluffs in general show evidences of deposition by river surges, and are marked by alternating layers of coarse sands with cobbles and silty clay. The coarser layers may have been deposited during the floods and fine sediments during the leaner seasons. Texturally, the sediments can be classified as clay loam, sandy clay and silty clay loam (Figure 3). These types of sediments have been noted for major rivers in Maharashtra, and are categorized as flood loams or diluvium[5]. Studies in upland Maharashtra show that the modern fluvial depositional processes in the region must have been initiated in the late Pleistocene–early Holocene[5]. These rivers are noted for highly fluctuating discharge and active channel migration[6]. The period of the initiation of fluvial deposition at Ter is, however, uncertain. An exploratory pit at the base of bluff indicates that the alluvium extends (>2 m) further down below the present river bed. This probably suggests that fluvial processes at Ter must have started much earlier, analogous to other rivers in Maharashtra.

Because of the relative accessibility and fuller development of sedimentary units, a representative section, trending roughly in the north-south direction was selected for the present study (Figure 1 *b*). Apparently, the depositional layers in the section seem to have been disturbed by anthropogenic features indicating human intervention. The settlers had made strong pebble foundation, maybe as a precaution against floods. Three succeeding layers of reddish ceramics with remnants of pebble foundation at different elevations could be detected which may belong to three different periods. It is probable that each settlement had been destroyed by floods and covered by succeeding river deposits, which was again intermittently occupied at different periods. Remnants of these settlements occur at an elevation from the present-day river channel.

The sedimentary units observed in the section on the whole have been warped at different scales. On a large scale, the entire section appeared to have been flexed. Individually, the structures present in the section can be broadly categorized as flexures, warps and buckle folds (Figure 4 *a*, *b*). The structures including warping and low-amplitude folding of near-surface beds of alternating mud and cohesionless sediment have been reported from the other earthquake-prone areas also[7]. It was observed that the structures at Ter are better developed on the more competent layers such as the layers containing ceramics and pebble foundations made by the settlers, although weak traces of folds can be discerned on the argillaceous layers as well, on closer look. Variation in the intensity of deformation observed in individual layers that belong to the same deforming mass can be explained by strain partitioning, which depends on the bulk properties of the rock[8]. Because of partitioning of mechanical behaviour, the stiffer and more competent rocks are expected to show variation in shapes and wavelengths of folds. From the style of deformation, it is clear that the structures in section at Ter have been formed by buckling of the sediment strata. Buckling must have been preceded by layer parallel shortening, and accompanied by flexural slip between the layers (Figure 4 *a*, *b*). The pattern of deformation such as flexures and associated shortening, which is evidently the dominant pattern of deformation in the Ter section, may

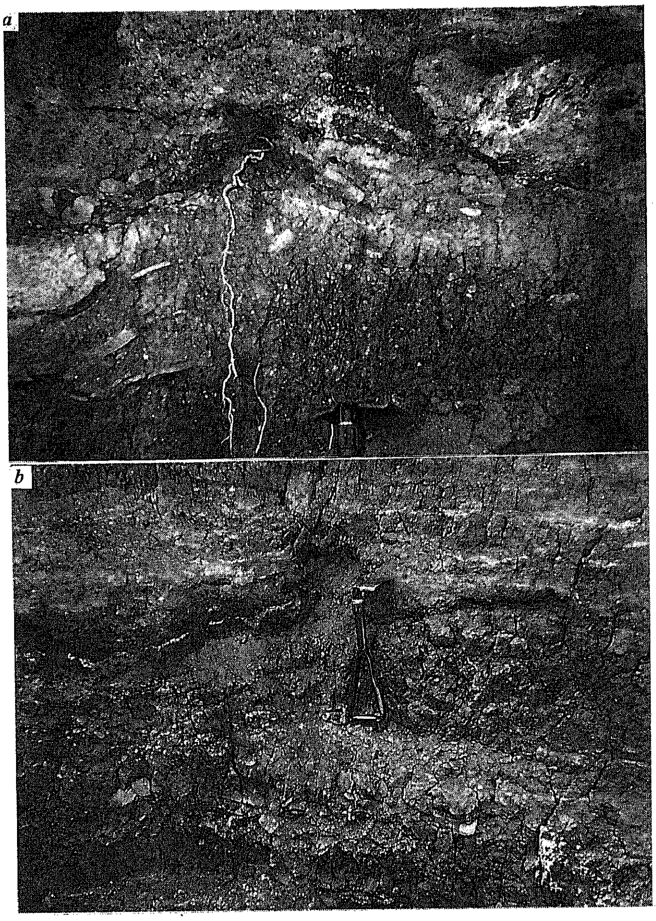

Figure 4. *a*, Photograph of the middle of the section showing flexure and shortening; *b*, Photograph of the section, 2 m below the surface showing warping of sediment layers.

Table 1. Radiocarbon dates of charcoal samples from the sedimentary section at Ter, Osmanabad District, Maharashtra

Lab No.	Sample	Measured ^{14}C age@	Calibrated B. P.†# Age	1σ age range
BS-1213	1/T	2300 ± 90	2220	2340–2110
BS-1214	2/T	2360 ± 110	2330	2390–2150
BS-1215	3/T	2150 ± 80	2030	2140–1940
BS-1233	4/T	1730 ± 150	1560	1750–1380
BS-1217	01/T	2170 ± 100	2070	2180–1940
BS-1232	11/T	2170 ± 100	2070	2180–1940

Ages are rounded off to nearest 10 yr.

@ ^{14}C ages calculated using the half life value 5730 ± 40 yr.

Calibration based on marine ^{14}C age data set using the radiocarbon calibration program Rev. 3.0.3 (ref. 21).

† σ^{13}C values are assumed as –25 ± 2 per mil (value for wood charcoal).

have been generated in a stress field where the maximum principal compressive stress is horizontal and the minimum stress is vertical[9].

Apart from the aforementioned structures, the vertical offsets of marker horizons at the northern part of the section (Figure 5 *a*, *b*) indicate a displacement of 10–15 cm. The upthrown block is on the southern side of the offset plane. The offset, extending downward, can be easily detected in the repeated sequences of 15–20-cm-thick interlayers of whitish clay, occurring in a predominantly blackish clay (Figure 4 *a*). Further, the structures consisting of load casts and contorted lamination were recorded along the western bank of River Terna. These structures are confined to a horizon occurring 1 m below the surface, separated by flat lying undeformed beds. It appears that the sand-rich unit was liquefied and sagged into the clay-rich host material. Development of similar structures associated with seismicity elsewhere has been discussed by other workers[10, 11].

The horizontal shortening revealed in the sedimentary layers (Figure 5 *a*) conforms to the direction of applied stress in northeast-southwest direction, which is the direction of $Sh_{\max}$ in the region[12]. This is a critical factor which helps to confirm whether seismic processes are responsible for generating these features. The regional pattern, orientation and abundance of these features and their overall conformity with the direction of maximum compressive stress point to the predominant role of seismic processes in the deformation of the sedimentary layers.

The radiocarbon dates of the samples obtained from the section are given in Table 1. The age obtained from the top layers (Figure 1) is 1560 yr B.P. and the age for the bottom layers ranges from 2330 to 2070 yr B.P. The actual age of causative event can be constrained only when both maximum and minimum ages from the confining layers on the top and bottom of the event horizon are available[13]. The radiocarbon dates ranging from 2330 to 2070 yr B.P. represent maximum age of the deformation event. Because the deformed layers do not extend to the layers deposited above the third settlement, the minimum age of the deformation event may be around 1560 yr B.P. The ^{14}C dates generally conform to the ages deduced for the ancient settlements based on the archaeological data[14–16], which suggest three major periods of settlements: episode I: ~2300 years; episode II: ~2000 years; and episode III: ~1600 years. The final period was culturally the most active during which time some of the major structures including the *Trivikramesvara* Temple (350–450 A.D.) were presumably built.[14] After that, the area appears to have been abandoned until the medieval period[14]. Based on the above inferences, it is postulated that the seismic event must have occurred sometime during 350–450 A.D.

However, the monuments at Ter outwardly appear to be intact. It is difficult to assume that the structures built 1500 years ago had in fact withstood a damaging earthquake that occurred during the same interval. These contradictory factors, viz. the evidence of tectonic disturbances in the sedimentary section on one hand, and the

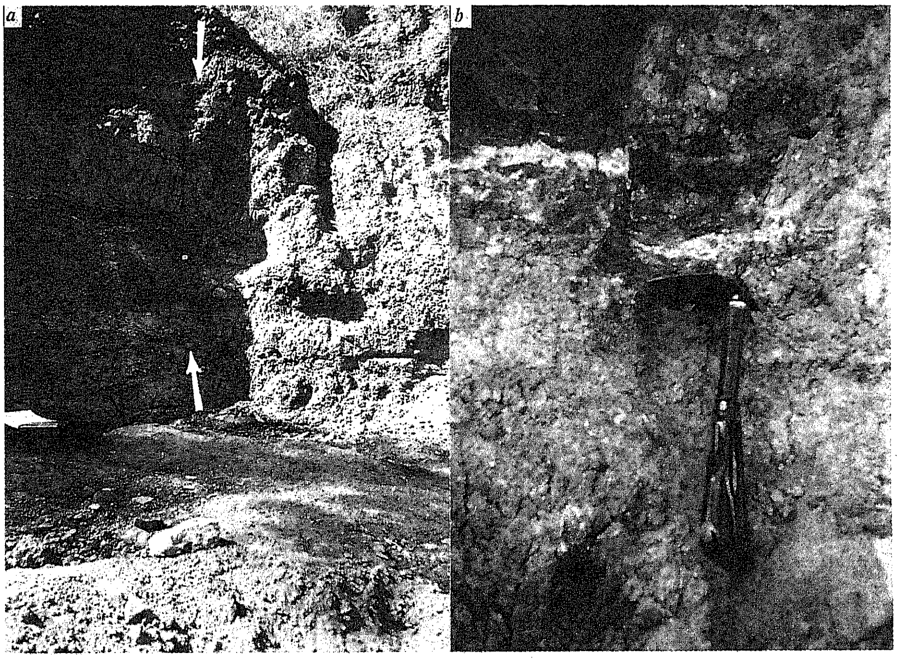

Figure 5. *a*, Part of the section showing offset; *b*, Close-up of the offset in the middle of the section.

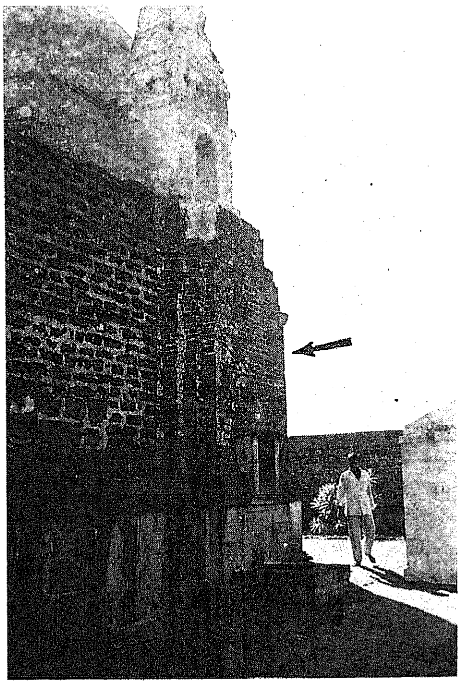

Figure 6. Photograph of a temple at Ter. Note the older basement and later reconstruction (indicated by an arrow) over the basement.

apparent insulation of monuments to the seismic event on the other, are to be reconciled in order to obtain a logical conclusion. The answer to this puzzle probably lies in a revealing observation of Cousens[14], on the most important monument of Ter, namely, the *Trivikramesvara* Temple. This structure, which essentially consists of a shrine and a *mandapam*, according to Cousens[14], was a rebuilt one. Based on compelling architectural signatures, it is suggested that they were rebuilt in medieval period[15], a time far removed from the period in which it was originally constructed. In fact, my inspection suggests that not only the *Trivikramesvara* Temple, but all the historical monuments at Ter without exception appeared to have been stripped off at the level of basement originally and were reconstructed in the styles that were in vogue in the later periods (see Figure 6). The structural damages must have been caused by the earthquake that occurred around 1500 years ago.

It is worthwhile to note that the $Mw = 6.1$ 1993 Killari earthquake, which released a moment $Mo = 1.8 \times 10^{18}$ nm from a reverse fault rupture, is characterized by an average displacement of 2.1–0.8 m and a stress drop of 160–45 bars[17]. It has also been shown, based on aftershock distribution, that rupture area of the 1993 Killari earthquake is fairly small (38.5 km^2) involving a fault length of only 5.5 km (ref. 18). These data related to the Killari earthquake, one of the rare cases of a well-documented stable continental earthquakes, is important in assessing the probable dimensions of the ancient earthquake occurred at Ter which has analogous geological characteristics. The offset (15–20 cm) in the sedimentary section at Ter reflects only a fraction of movement in the basement fault below the basalt layers. Based on the experience of the recent example of Killari event, the inferred extent of the damage intensity

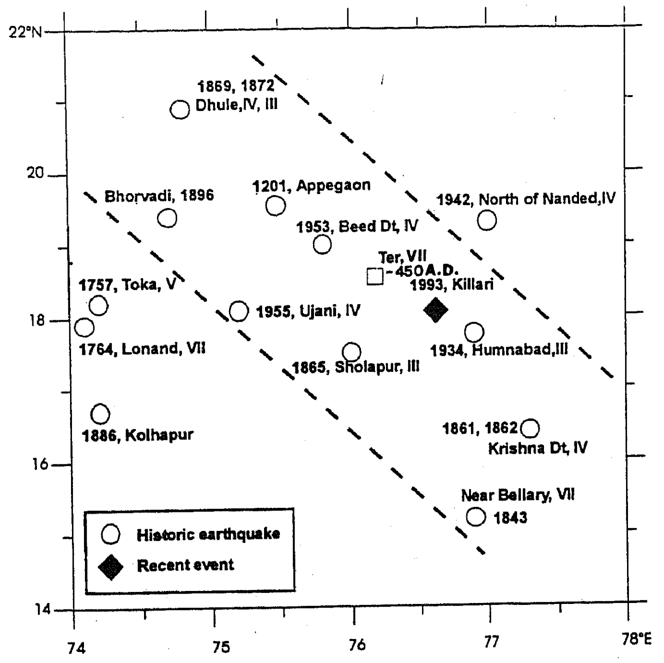

Figure 7. Location of historic earthquakes (A.D. 1201–1960) in the region. Site at Ter forms a part of the northwest-trending structure.

(defined by the count and spread of affected monuments) and the projected displacement in the basement rock from the surface measurements, it is inferred that the ancient earthquake of Ter had a magnitude similar to the 1993 Killari event with a meizoseismal area that must have been narrow and spatially restrictive.

The sedimentary sections, located on an elevated ground, are now much above the present river bed. Obviously, the question is how to account for such an abnormal elevation compared to the flat-lying country surrounding the bluffs at Ter. The present-day elevation of the section (10 m from the river bed) could not have been accounted entirely by the actual coseismic slip during a single earthquake that occurred ~1500 years ago. Taking a cue from the 1993 Killari earthquake, the actual offset in the basement for a similar earthquake at Ter could not have been >1–2 m. Because of the fact that the major part of the deformational structures in the section appears to be related to folding, it is reasonable to assume some amount of previous flexural slip on the underlying basalt layers. It has been demonstrated by several examples elsewhere in the world that the folds form as a result of repeated thrust events in some tectonic environments[19,20]. Our studies in the 1993 Killari rupture zone confirmed the occurrence of repeated thrust events on a pre-existing fault which is manifested in the near surface by parallel folding[1–4]. Having been reactivated several times in a compressive stress regime, some zones along this pre-existing regional fault may be conducive for development of thrust sheets in the basalt layers. Therefore, it is likely that even before the last earthquake some amount of topographic high was maintained in the basalt flows at Ter due to accumulated slip produced possibly by the earlier earthquakes. However, since no accumulated slip was observed in the sedimentary section, it is assumed here that only one earthquake occurred during the last 2000 years – the interval during which the sediments were deposited (see Table 1, for age constraints). It is concluded that the original uplift may have been cumulatively accentuated by the incision of the river, deposition during the floods, coseismic slip during the last seismic event and erosion of river bank, maintaining the present-day topography.

Our studies showed that the 1993 Killari (Latur) earthquake, although initially considered as an unprecedented event[17], is closely associated with the continuing seismogenic processes in the region, and was not an isolated event on a discrete fault[1–4]. The studies also demonstrated that the 1993 event occurred on a fault that is part of a northwest-trending regional structure. The spatial distribution of historic earthquakes and the sites including Killari and Ter, defining a northwest-trending corridor, extending as far as >400 km suggest the reactivation potential of this fault zone (Figure 7). One important consequence of the present study is the realization that the earthquake that occurred at Ter and the recent one at Killari may not be isolated events, but associated with this active northwest-trending regional structure.

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Deccan Trap dioritic gabbros from the western Satpura–Tapi region

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Large (up to 3 m), xenolith-like boulders of dioritic gabbros are found in the Deccan basalts near Shahada and Shirpur in northern Maharashtra, along ENE–WSW-trending structural ridges. Both these dioritic gabbros have been dated by the ^{40}Ar–^{39}Ar incremental heating technique, and have yielded typical Deccan ages of ~ 66 Ma. These ages rule out the possibility of these rocks representing the basement crust of the area. The rocks from both ridges are petrographically, chemically and isotopically similar. In addition, their Sr–Nd–Pb isotopic ratios indicate that they are isotopically identical with some of the Mahabaleshwar Formation lavas of the Western Ghats Deccan. However, these rocks are more evolved chemically compared to typical Mahabaleshwar Formation lavas. These characteristics suggest that they are the likely products of fractional crystallization of a parental Mahabaleshwar-like magma generated in this region.

DURING our field mapping of Deccan basalts of the Western Satpura hills and Tapi river valley in parts of Dhule district, northern Maharashtra, we have come across large blocks of dioritic gabbros appearing to be xenoliths contained in the basalts. We have noted two such occurrences; the first about 7 km south of Shahada (21°35'N, 74°30'E), and the other about 20 km north of Shirpur (21°20'N, 74°50'E) (Figure 1).

The Shahada dioritic gabbros are seen exposed as large loose boulders, up to 3 m in diameter, along an ENE–WSW-trending low ridge of basalts. This ridge begins 7 km south of Shahada, at the bifurcation of roads to Dondaicha (21°20'N, 74°35'E) and Shirpur, and runs parallel to the Shirpur road for a further 6 km. After 6 km the ridge abruptly disappears, and the area is covered with alluvium of the Tapi river. The ridge is made up of a plagioclase-phyric, two aphyric and one giant plagioclase basalt (GPB) flows and many small basaltic dikes.

The other occurrence of such boulders is at Nandarde village near Boradi (21°32'N, 74°53'E), 20 km north of Shirpur. These are seen along an ENE–WSW ridge about a km in length, which is made up of an aphyric basalt flow and a GPB compound flow. Many small dike exposures are also found.

Interestingly, the Shahada and Nandarde ridges seem to lie along a single ENE–WSW lineament (coinciding with the Narmada–Satpura–Tapi regional tectonic trend), reflecting some prominent, > 30 km-long fault structure, though physical continuity between them is lacking due to the widespread occurrence of Tapi alluvium. The flow stratigraphy for the ridges is also not the same.

Petrographically, chemically and isotopically also, the dioritic gabbros from the Shahada and Nandarde ridges are very similar. They are coarse grained with subophitic texture, and their main minerals are sodic plagioclase (An_{10-30}, i.e. oligoclase) and diopsidic augite. Some grains of orthoclase and labradorite are also present. Secondary hornblende forms a minor phase mantling the margins of some augite grains, and a few subhedral-sieve textured opaques are seen. The chemical and isotopic compositions of these rocks are given in Table 1.

For us an important problem was to decide whether the dioritic gabbros are related to the basalts, or whether they represent the older, possibly Precambrian, basement crust for the Deccan volcanics in this area. We considered the latter possibility quite likely, because

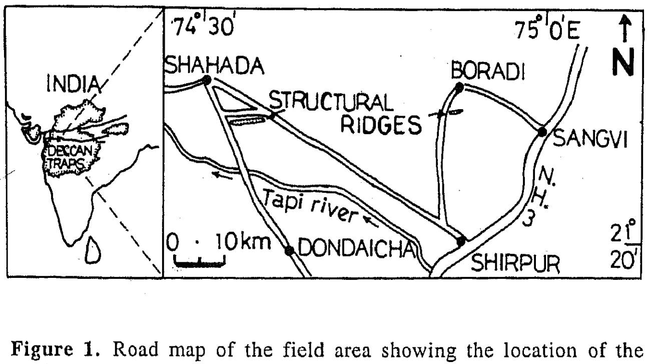

Figure 1. Road map of the field area showing the location of the Shahada and Shirpur ridges.

Table 1. Chemical and isotopic compositions of the dioritic gabbros

Wt. %	Shahada (SH 43)	Nandarde (SH50)
SiO_2	52.85	50.09
Al_2O_3	12.37	12.37
TiO_2	3.23	2.96
FeO (Total)	16.07	14.68
MnO	0.20	0.19
MgO	3.47	3.82
CaO	8.46	8.56
Na_2O	2.53	2.76
K_2O	0.95	0.90
P_2O_5	0.61	0.53
LOI	0.46	2.00
Total	101.20	98.96
ppm :		
Co	8	7
Ni	28	21
Cr	32	38
Sc	35	38
V	345	450
Ba	142	114
Sr	221	239
Zr	275	194
Y	62	43
Cu	209	192
Zn	196	191
Nb	21	17
Rb*	23.5	34.2
Nd*	32.03	71.14
Sm*	8.31	10.96
Pb*	2.74	2.75
Sr*	212.6	230.5
Mg # (molecular)	31	35
Zr/Nb	13.1	11.41
Ba/Y	2.29	2.65
Zr/Y	4.44	4.51
Ba/Zr	0.52	0.59
ε Nd_T (T = 66 Ma)	+ 1.4	+ 1.5
$(^{87}Sr/^{86}Sr)_T$	0.70481	0.70474
$(^{206}Pb/^{204}Pb)_0$	17.483	17.578
$(^{207}Pb/^{204}Pb)_0$	15.366	15.379
$(^{208}Pb/^{204}Pb)_0$	38.144	38.326

1. Mg # (Mg number) is molecular, assuming that 85% of the FeO (total) is in the FeO form. So Mg # = wt.% MgO/[MgO + 0.85 FeO Total] × 100.
2. LOI is loss in weight of sample powder on ignition at 900°C for about 2 h.
3. All major and trace element abundances have been measured by inductively coupled plasma – atomic emission spectrometry (ICP-AES) at the Regional Sophisticated Instrumentation Centre, IIT Bombay.
4. Rb, Nd, Sm, Pb and Sr (marked*) have been measured by the isotope dilution technique at the University of Hawaii. Analytical uncertainty on Nd and Sm abundances is $< 0.2\%$, on Sr $< 0.5\%$, on Rb ~ 1%, and on Pb $< 1\%$. Small acid-washed chips of the samples were used for this (as part of the procedure for isotope ratio determinations, e.g., see ref. 8), and hence the isotope dilution abundance values are not strictly representative of the bulk rocks, and have been used only to age-correct the Sr–Nd isotopic ratios. However. the isotope dilution abundances for Sr are very close to the whole-rock (ICP-AES) Sr abundances.
5. ε Nd_T and $(^{87}Sr/^{86}Sr)_T$ are the initial ratios age-corrected to 66 Ma. Pb isotopes are present-day values.
6. Analytical uncertainty on ε Nd is better than ± 0.2 units; on $^{87}Sr/^{86}Sr$ better than 0.00002; on $^{206}Pb/^{204}Pb$ and $^{207}Pb/^{204}Pb$ better than ± 0.012, and on $^{208}Pb/^{204}Pb$ better than ± 0.038.
7. Data are reported relative to the following standard values: For NBS 981 Pb, to the values of ref. 9. For NBS 987 Sr, $^{87}Sr/^{86}Sr = 0.71024 \pm 0.00002$. For La Jolla Nd, $^{143}Nd/^{144}Nd = 0.511845 \pm 0.000010$, corresponding to an analytical uncertainty of 0.2 ε units.

Table 2. ^{40}Ar–^{39}Ar radiometric ages of Deccan dioritic gabbros

	Age spectrum		Isochron			
Sample	^{39}Ar (%)	Age ± 1 s (Ma)	Age ± 1 s (Ma)	Intercept ± 1 s	$\frac{\text{SUMS}}{(N-2)}$	N
SH43	91	65.6 ± 0.5	64.4 ± 1.8	297.7 ± 5.9	2.59	5
SH50	88	65.6 ± 0.6	65.1 ± 0.7	291.5 ± 5.0	1.06	5

Ages calculated using the decay constants, $\lambda_\alpha = 0.581 \times 10^{-10}$ yr^{-1}, $\lambda_\beta = 4.963 \times 10^{-10}$ yr^{-1}, and biotite monitor FCT-3 (27.7 ± 0.2 Ma).

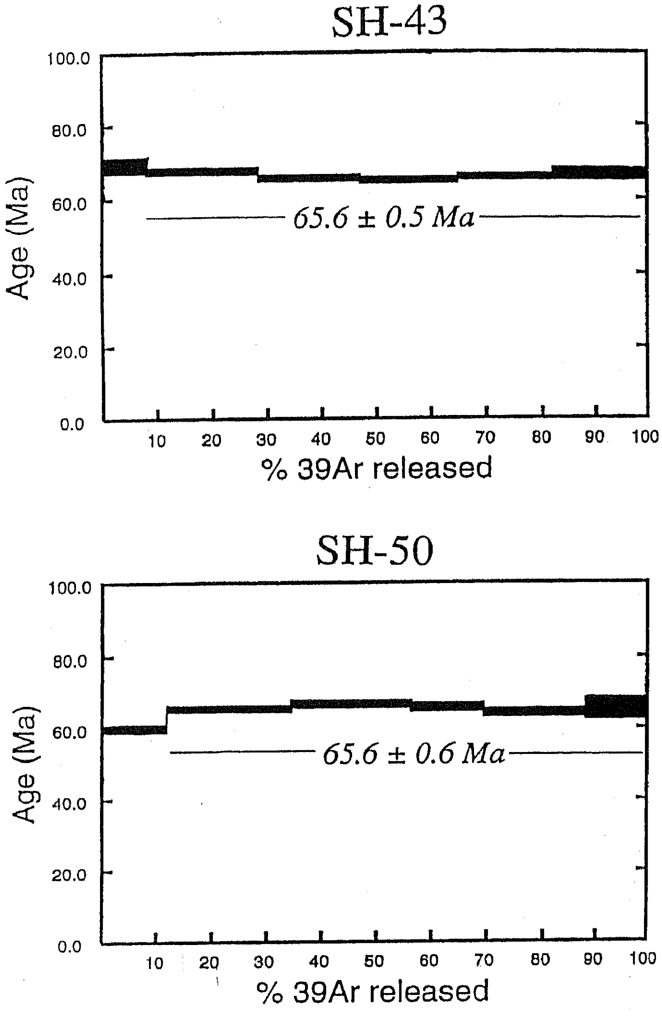

Figure 2. Plateau age plots for the Shahada (SH43) and Nandarde (SH50) dioritic gabbros.

such uplifted blocks of Precambrian granitoid rocks (such as monzonites) have previously been described by Chandrasekharam and Viladkar[1] from Mandaleshwar on the Narmada river. Surendranath[2] has also reported inliers of Precambrian granitoids from several places in the Tapi valley. However, radiometric dating by the ^{40}Ar–^{39}Ar incremental heating technique at Oregon State University yielded typical Deccan ages of ~ 66 Ma (million

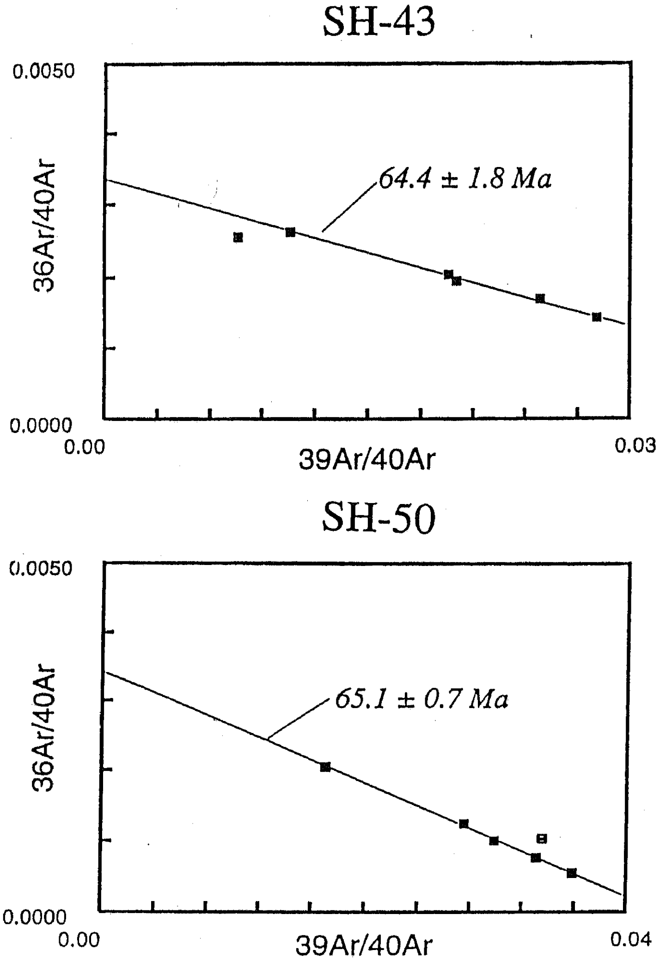

Figure 3. Isochron plots for the dioritic gabbros.

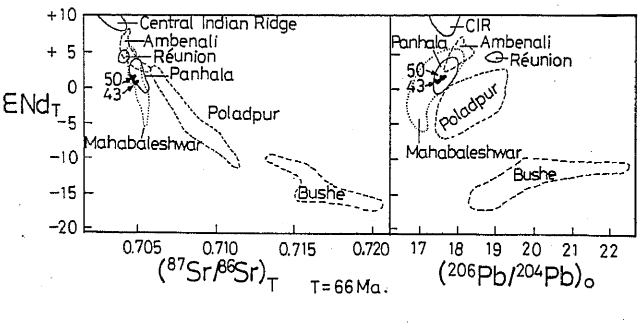

Figure 4. Nd–Sr–Pb isotopic plots showing the affinity of the dioritic gabbros with the Mahabaleshwar Formation of the Western Ghats Deccan Traps. Formation fields are after ref. 10.

years) for both samples (Table 2). The analytical procedures are described in Duncan and Hogan[3]. The Shahada sample (SH43) has been dated at 65.6 ± 0.5 Ma and the Nandarde sample (SH50) at 65.6 ± 0.6 Ma (Figures 2 and 3). The analytical errors are quoted at the 1–σ level, and are referenced to the biotite monitor FCT-3 (27.7 ± 0.2 Ma). These dates prove that the dioritic gabbros are related petrogenetically to the Deccan basalts.

As concerns the petrogenesis of the dioritic gabbros, their slightly positive ε Nd_T values indicate derivation from a slightly depleted mantle source. Interestingly, on plots of ε Nd_T vs $(^{87}Sr/^{86}Sr)_T$ and ε Nd_T vs $(^{206}Pb/^{204}Pb)$ (present-day), these rocks plot within the isotopic array of the Mahabaleshwar Formation from the Western Ghats[4] (Figure 4). The genetic links of these rocks with a Mahabaleshwar-like magma become clear on comparing their Nd–Sr–Pb isotopic ratios with those of typical Mahabaleshwar Formation lavas[5–7]. However, these rocks have lower Mg # and compatible element abundances (Ni, Co, Cr), and higher incompatible element abundances (such as Ba, Zr) than the Mahabaleshwar Formation lavas. These features suggest the likely origin of these rocks by the fractional crystallization of a parental Mahabaleshwar-like magma generated in this region. This fractional crystallization, however, was arrested at an early stage as evidenced by the transitional character of these rocks between true gabbros and true diorites. These rocks are considerably more evolved than normal gabbros (e.g., with low Mg numbers and evolved plagioclase compositions). At the same time, they are not true diorites as they lack primary hornblende, the most characteristic ferromagnesian mineral of true diorites.

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Princeton Guide to Advanced Physics. Alan C. Tribble. Princeton University Press, 41, William Street, Princeton, New Jersey 08540, USA. 1996. 397 pp. Price: $ 59.50, Clothbound.

It is widely acknowledged that the ability to solve problems is a most essential part of higher education in physics. Dissatisfaction with standard university curricula in our country in this regard is equally widespread. For a variety of reasons, our 'system' is simply unable to instil any notable degree of skill in problem-solving in students who are awarded B Sc and M Sc degrees in physics.

In this situation, any effort that helps propagate the problem-solving culture must be welcomed. Over the years, a number of collections of problems with solutions have appeared. Restricting oneself to the graduate level, and leaving out collections on specialized subjects (especially quantum mechanics), mention may be made of *University of Chicago Graduate Problems in Physics* by J. A. Cronin *et al.* (Addison-Wesley, 1967); *University of California, Berkeley Physics Problems* by M. Chen (Prentice Hall, 1974; Prentice Hall India, 1990); *Problems in Theoretical Physics* by L. G. Grechko *et al.* (Mir, 1977); and *Princeton Problems in Physics* by N. Newbury *et al.* (Princeton University Press, 1991). There are undoubtedly several other collections of this sort, covering all the standard subjects – classical and quantum mechanics, statistical physics, electromagnetism, etc. I opened the book under review with the expectation that it might be another such collection, but it is actually a 'comprehensive one-volume reference book' as proclaimed on the cover. The blurb on the back cover starts by saying, 'From classical mechanics to general relativity, the key principles in all areas of physics are surveyed in this one handy volume'. Indeed, the 12 chapters of the book do range over a wide pasture – mathematical methods, classical mechanics, electrodynamics, etc. Brief derivations are given of standard basic formulas and material in each subject, with some illustrative examples. The book reads like the set of note-books a graduate student preparing for a comprehensive exam is likely to build up – just the essentials, no deep insights, somewhat random in the detailed choice of topics, and hence somewhat disconnected; in short, no different from what we in this country would succinctly categorize as a guide rather than a textbook.

Now, a guide that is not too bad could actually be more effective than a combination of poor teaching and a textbook that is either bad, or is written at too inaccessible a level for the students concerned. The guide could then help build up the student's self-confidence and open the doors to more reliable and systematic sources of information. However, one must allow for the fact that guides are generally non-rigorous and superficial, and could be often misleading and occasionally downright wrong. The question, therefore, is: how reliable a guide is the one under review? It is, after all, published by Princeton University Press – an establishment whose output in physics and mathematics is known to be of the highest standards of excellence.

Unfortunately, I find that the book is not up to the mark. The number of incorrect statements scattered all over the book is simply too high to be acceptable, and often of a seriousness too great to be dismissed as arising out of carelessness or informality of presentation. Facile definitions and incomplete statements may be acceptable under the 'guide' framework, but totally erroneous ones cannot be condoned. Leaving aside even serious typos, let me list a few of the glaring errors I found on merely flipping through the pages of the book, to let readers judge for themselves:

p. 4: $\delta(x - x')$ is defined to be equal to 1 when $x = x'$.

p. 26: For $f(z)$ *analytic* in a neighbourhood of $z = a$, and C a simple closed path surrounding a, it is claimed that $\int_C f(z)\mathrm{d}z = 2\pi i$ Res $f(a)$. This is called the residue theorem! It is then *followed* by what happens when $f(z)$ has an isolated singularity at $z = a$, and it is claimed that Res $f(a) = \lim_{z \to a} (z - a) f(z)$

for *any* isolated singularity at $z = a$ (!).

p. 30: In a section on tensors, the coordinates are introduced as covectors rather than vectors, and 'equations' such as

$g_{ij} = \partial x_j / \partial x_i'$, $A'^i = g_{ij} A^j$, etc. are written down, with absolutely no regard for the distinction between upper and lower indices, in a presumably arbitrary space!

p. 37: The discussion of the 2-body central force problem suggests that the author has simply equated $(\mathrm{d}\vec{r}/\mathrm{d}t)^2$ with $(\mathrm{d}r/\mathrm{d}t)^2$. It is also stated that a central force between two particles implies 'an interaction potential V, where V is any function of the vector between the particles' and V is subsequently written as $V(r, \dot{r} \ldots)$!

p. 39: The area swept out by the radius vector of a particle in the Kepler problem is claimed to be $A = (1/2)\ r\ (r\ \theta)$!

p. 84: $\left| \vec{R} - \vec{r} \right|^{-1}$ is expanded as

$$(1/R) \sum_{n=0}^{\infty} (r/R)^n P_n(\cos\theta)$$

for arbitrary *(but finite)* R and r *!*

p. 194: Having used $|\psi\rangle$ to denote the *general* state of a system, the author goes on to say: 'If one measures the observable corresponding to the operator A and finds the value λ_n then immediately after the measurement the state vector satisfies $i\hbar\, \partial|\psi\rangle/\partial t = H|\psi\rangle$. We say that the measurement projects the original state vector onto eigenvectors (*sic*) of A.'

p. 198: '. . . quanta of matter or radiation must be represented by wave packets. For this reason the state function ψ is also called a wave function. That is, ψ must have properties of plane waves, $\psi \propto \exp\ i(kx - \omega t)$.'

p. 225: The eigenvalues of $L_z/\hbar$ are integers, it is claimed, because the wave function and its derivative 'must be continuous over the range 0–2π' of the azimuthal angle!

One could go on, but here's a good punchline:

p. 307: 'All mesons are unstable *and have no distinct antiparticles.*'

Need one say more? Book-writing is (or at least ought to be) a sobering exercise for both authors and publishers. It brings out so tellingly the need for painstaking attention to the smallest detail. This is all the more important in a society where students (and also administrators!) regard the printed word as sacred. In the spirit of these quotations, let me end with a memorable quote from a well-known elementary text on materials science. It is not my intention to add gratuitous insults, but to bring out the perils attendant upon attempting broad definitions without adequate care. In *Vol. I: Structure* by W. G. Moffatt, G. W. Pearsall and J. Wulff, of the 4-volume text *The Structure and Properties of Materials* (Wiley, 1964; Wiley Eastern, 1974), at the end of the very first chapter (on electrons and bonding), one finds a set of 'definitions'. Among them are:

'Quantum mechanics: A branch of physics in which the systems studied can have only discrete values of energy, separated by forbidden regions; it is distinguished from continuum mechanics in which a continuum of energies is assumed possible.'

'Wave mechanics: A branch of mathematical physics involving the statements and solutions of differential equations describing wave behavior.'

Who says science is humourless?

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Myocardial Ischemia: Mechanisms, Reperfusion, Protection. M. Karmazyn, ed. Birkhauser Verlag, P.O. Box 133, CH – 4010, Basel, Switzerland. 1996. 528pp. Price: SFR 198, DM 238.

Myocardial infarction is one of the most common causes of hospital deaths. Thirty years ago it was generally accepted that insufficient blood flow due to coronary artery obstruction by atherosclerotic process is the cause of myocardial ischemia and that infarction is precipitated by occlusion of coronary artery by thrombus. Therapeutic approaches were aimed at thrombolysis and limiting the infarct size. Recent clinicopathological, angiographic and experimental studies indicate that additional structural and functional alterations are superimposed on chronic altherosclerosis and these are responsible for the development of various ischemic syndromes. Considerable clinical and experimental data have accumulated which make apparent that prognosis following infarction depends on the amount of residual viable normally functioning mycardium and that interventions which would decrease the extent of tissue death will reduce mortality in patients. It has also been recognized that restoring blood flow to the ischemic mycardium is not always associated with recovery but may lead to further damage, recognized as reperfusion injury.

Karmazyn has edited a book which reflects the growth of understanding of pathophysiological mechanisms in acute myocardial ischemia and the current approaches to define rational therapeutic strategies to salvage ischemic myocardium. The book contains 31 articles grouped in 9 sections with distinctive titles. The contributors are all internationally recognized investigators.

The first, third and fifth articles are on nitric oxide. Zhao, Hintze and Kaley examine evidences for mediation of reflex coronary artery dilatation in ischemic conditions by nitric oxide produced by vascular endothelium. Several studies have earlier shown that activation of sympathetic or parasympathetic nervous system controls the resistance of coronary arteries. Bezold–Jarish reflex, carotid chemoreflex, arterial baroreceptors and ventricular receptors participate in this regulation. The observation that selective impairment of vagal control of coronary blood flow is due to the impairment of nitric oxide synthesis suggests that nitric oxide is a mediator of neural regulation of coronary vascular resistance.

Dusting provides a more extensive review on nitric oxide; its biosynthesis, role in inflammation and pathological conditions such as atherosclerosis, reperfusion injury and heart failure. Studies have demonstrated that atherosclerosis leads to disturbances in the activity of isoforms of nitric oxide synthase in the artery and can contribute to vasospasm, thrombosis and cell proliferation. These defects may be reversed, thus offering a promising target for therapy against some manifestations of vascular diseases. There is also evidence that derangement in nitric oxide function is associated with the development of atherosclerosis and vascular remodelling after angioplasty. Production and actions of nitric oxide are also abnormal in chronic heart failure. Nitrate, the stable end product of endogenous nitric oxide production is elevated and endothelium dependent vasodilatation impaired in patients with heart failure and the levels are proportionate to the severity of heart failure. The causes for enhanced release of nitric oxide from vascular endothelium and its functional consequences are still unclear.

Pabla and Curtis survey experimental studies in animals which imply a role for endogenous nitric oxide as a mediator for cardiac protection in atherosclerosis, myocardial infarction, reperfusion induced arrhythmias and coronary artery restenosis after angioplasty. Studies using inhibitors of nitric oxide synthase suggest that reduction of nitric oxide production exacerbates the disease state. Probably, to protect cardiac function in disease conditions, it may be of value to mimick nitric oxide function or induce its synthesis or block its degradation. Interestingly, in experimental animals short term exercise training increases the production of nitric oxide in the coronary vasculature and enhances nitric oxide synthase gene production in endothelial cells. Nitric oxide dependent coronary vasodilatation is also improved. These mechanisms may be responsible for improvement of cardiac function and reduction in incidence of coronary diseases, seen in patients who undergo exercise training.

Myocardial ischemia and reperfusion are associated with increased production of another endothelium derived peptide, endothelin. Reperfusion of ischemic myocardium is a potent stimulus for increased release of endothelin as is evident by elevated plasma levels of endothelin in patients with coronary artery disease who have had thrombolytic therapy or angioplasty. Elevated plasma levels are associated with diminished ventricular function and increased mortality. A number of investigations have been carried out in animals to assess the effects of endothelin receptor antagonists and other inhibitors in ischemia and reperfusion. Results imply that modulation of endothelin synthesis or its actions could represent novel therapeutic stategies. The topic is exhaustively dealt by Karmazyn.

Oxygen-free radicals have been extensively examined as potential mediators of injury in ischemia and during reperfusion. Free radicals can cause cellular dysfunction directly by oxidation of cell structure components. Another mechanism which has gained attention in recent years is alteration or destruction of second messenger pathways in the cell which affect cellular ionic homeostasis, contractile function and viability. Czubryt, Panagia and Pierce discuss the studies which address the deleterious effects of oxidants on various components of the second messenger system. The second messengers are important for regulation of normal cardiac function. Changes that occur in the signal transduction system during ischemia and reperfusion have possibly a role in arrhythmogenesis and other functional abnormalities. Recent evidences that reactive oxygen species and nitric oxide are linked to cellular second messenger system and that non-receptor mediated signal transduction is important as a mediator of ischemic and reperfusion injury point to new levels for pharmacological intervention.

Pettersson, Ostlund Lindquist and Westerlund in their article focus on experimental investigations which assess the antiatherosclerotic effects of probucol, a lipophilic and an antioxidant compound. Studies in Watanabe heritable hyperlipidemic rabbits as well as animals in which atherosclerotic disease was induced by hyperlipidemic diet reveal that antioxidants may inhibit development of lesions in aorta. However, it is not clear whether antioxidants can cause regression of pre-existing lesions. Also, there are no reports on the effects of antioxidants on progression or regression of coronary artery atherosclerosis either in animals or man. In animals, antioxidant therapy can prevent restenosis following angioplasty and also restore endothelial regulation of vascular tone which is impaired in atherosclerosis.

Myocardial dysfunction in ischemia and subsequent reperfusion result from a multitude of cellular abnormalities involving ionic homeostasis, metabolism and bioenergetics. Altschuld reviews calcium transport systems of mitochondria and sarcoplasmic reticulum and summarizes how they are altered during ischemia and reperfusion. Damage to mitochondria consists of alterations in the permeability of mitochondrial inner membrane which leads to uncoupling of oxygen consumption from the phosphorylation of ADP. In the sarcoplasmic reticulum, changes in the calcium efflux channels or ryanodine receptors could be responsible for contractile abnormalities.

Soon after the discovery of potassium channels, the potential link between ischemia induced potassium efflux from myocytes and ATP sensitive potassium channels (K_{ATP}) was realized. Modulators of potassium channels are being used for treatment of myocardial ischemia. Grover reviews the data which denote that K_{ATP} openers have potential to be used as protective agents in myocardial ischemia. However there is also the possibility that K_{ATP} openers may enhance arrhythmias.

Membrane phenomena at cellular and subcellular levels have been extensively explored, thanks to the availability of techniques such as NMR spectroscopy, voltage clamp measurements, etc. and use of channel blockers and fluorescent dyes. Alterations in potassium and calcium homeostasis which lead to reduced conduction velocity, a decrease in duration of the action potential and refractoriness, are important among the multiple cellular mechanisms invoived in ischemia-induced electrophysiological abnormalities. There are two articles which address electrophysiological responses and cellular mechanisms of arrhythmias in ischemic and reperfused heart.

Lemasters *et al.*, using cultured myocytes and perfused papillary muscles have probed for mechanisms, other than calcium overload, sarcolemmal injury and free radical damage, for loss of cell viability after reperfusion. Their results signify that reperfusion injury is linked directly to changes in intracellular pH. They hypothesize that naturally occurring acidosis during ischemia inhibits degradative enzymes such as phospholipases and proteases. After reperfusion, this inhibition is released as the pH returns to physiological levels and as a result plasma membrane gets damaged leading to cell death. The change in intracellular pH may also activate myofibrillar ATPase and cause permeability transition in mitochondrial membrane. Avkiran, in his article highlights the role of Na^+–H^+ exchanger which regulate intracellular pH and the possible therapeutic benefits of inhibition of the exchanger in myocardial ischemia and reperfusion.

The normal mammalian heart has an aerobic metabolism. According to energy demand, the rate of ATP production is regulated by pathways which use fatty acids, lactate and glucose as energy substrates. During ischemia, oxidative metabolism ceases and anaerobic glycolysis is stimulated. Other metabolic derangements also occur. These include inhibition of mitochondrial oxidative phosphorylation, reduction in the capacity to oxidize fatty acids, alterations in triacyl glycerol and phospholipid metabolism, and defective utilization of glucose and free fatty acids. There is accumulation of lactate, protons, nucleosides, inorganic phosphate and acyl carnitine. There is additional tissue injury during reperfusion. Das and Maulik discuss the impairment of bioenergetics in ischemia. Vander Vussue and colleagues detail lipid metabolism in ischemic heart and indicate the possible relationship between altered lipid metabolism and irreversible damage of myocytes.

Another review is centred on arachidonic acid metabolism and how alteration in the production of arachidonic acid metabolites can affect heart function in health and disease. Under ischemic conditions and during reperfusion, blood cells are activated and their migration and interaction with endothelial cells increase eicosanoid biosynthesis. Their precise functions are not known. Prostaglandins have been implicated as mediators of reperfusion associated dysfunction. Inhibitors of prostaglandin synthases improve ventricular function.

Cardiac injury in ischemia is often extended and complicated by inflammatory reaction. Leucocytes may also damage potentially viable myocardium especially when coronary blood flow is reestablished by reperfusion techniques. Frangogiannis, Youker and Entman elucidate the role of the neutrophil in myocardial infarction and propose a hypothesis which describes the events that mediate post-perfusion imflammatory injury. Attempts are being made to identify specific cellular and molecular targets for therapeutic intervention. Pharmacological approaches include the use of neutrophil antibodies, neutrophil depleting anti-metabolites, neutrophil filters, free radical scavengers, lipooxygenase inhibitors, agents to deplete complement, and leukotriene B antagonists. Prostacycline analogues and adenosine are being tried to alter neutrophil function. Recent studies suggest that monoclonal antibodies against CD 18 and adhesion molecules L-selectin and P-selectin are effective in reducing myocardial necrosis, preserve coronary endothelial function and attenuate neutrophil accumulation in ischemic regions.

Investigators the world over have found that if myocardium is subjected to brief periods of ischemia followed by reperfusion, myocardium develops resistance to infarction from a subsequent severe ischemic episode. This phenomenon is termed preconditioning. The protection includes reduction in cellular damage, ventricular dysfunction and arrhythmias. The mechanism of preconditioning is being intensively explored in numerous laboratories. Several studies are focused to find clues to design pharmacological approaches for preconditioning in patients with ischemic heart disease. Miura and colleagues present the current understanding about preconditioning mechanisms. Adenosine A1 receptor has a key role in preconditioning. Protein kinase C and ATP sensitive potassium channel are possibly important effectors.

Animal studies have revealed that protection in ischemic preconditioning is biphasic. There is an early, rapid onset but transient protection and a second delayed protective adaptation. Both are dependent on adenosine receptor activation. The delayed protection is also associated with increase in myocardial heat shock protein-HSP-70 and increase in activity of the enzyme superoxide dismustase. Interestingly, in experiments using myogenic cell lines transfected with a cDNA encoding human HSP-70, it has been demonstrated that a single stress-related gene product can protect cells against both lethal heat stress and simulated ischemia. There are also reports of improved post-ischemic functional and metabolic recovery or increased resistance of the heart to ischemic injury in transgenic mouse models which overexpress HSP-70. Heads, Latchman and Yellon in their article on the role of stress proteins and antioxidants in adaptation to ischemia, suggest that identification of the genes which are important in mediating ischemic preconditioning and determination of how their promoters are regulated will lead to possible gene therapies for myocardial ischemia.

Currently, there are several attempts to harness endogenous protective mechanisms and considerable knowledge has accumulated on the role of adenosine which has numerous cardiac effects. Cook and Karmazyn review the mechanisms implicated in cardioprotection by adenosine and the potential role of adenosine analogs as protective agents in ischemia. Cardioprotection from ischemia is important in preservation of donor hearts intended for transplantation, and during various cardiac operative procedures using cardiopulmonary bypass. An overview of approaches to myocardial protection in such conditions is provided by Myers and Fremes.

A major factor which influences survival of patients with ischemic heart disease is, ventricular function. Any improvement in ventricular function is associated with better survival. In about 42% of patients, abnormalities of ventricular contraction may not necessarily mean dead tissue or a scar. Ischemic tissue may downregulate its function and metabolism for survival. This adaptive phenomenon has been termed hibernation of the myocardium. Though the exact mechanism underlying hibernating myocardium remains controversial, both animal and human studies reveal that prompt establishment of blood flow improves function of the hibernating myocardium and survival of patients. A similar phenomenon is myocardial stunning. While hibernating myocardium occurs in a hypo-perfused heart that reduces its oxygen demand by decreasing contractility, stunned myocardium is a feature of an ischemic heart but with normal perfusion. Differentiation between stunned and hibernating myocardium is critical. Leor and Kloner review the current views on the hibernating myocardium. New non-invasive techniques for detecting and treating stunned myocardium are analysed by Allen, Cox and Kloner.

The last section of the book deals with the healing processes in the infarcted myocardium and the compensatory changes that occur in the remaining tissue. These alterations or remodelling occur at three levels, viz. in the chemical composition of the myocytes and extracellular matrix (biochemical and subcellular remodelling), in the microscopical structure (structural remodelling) and in the three-dimensional configuration of the ventricular chambers (geometrical remodelling). It is now realized that remodelling is mediated by the activation of sympathetic nervous system, circulating renin–angiotensin–aldosterone system (RAAS), locally generated tissue angiotensin II and formation of growth factors. Studies on remodelling in the infarcted heart are summarized in three articles.

Recognition that changes associated with ventricular remodelling are responsible for ventricular dysfunction in the post-ischemic period has prompted search for therapeutic strategies aimed at improving the healing process after myocardial infarction. Novel approaches are scrutinized by Jugdutt. Pharmacological agents which influence the inflammatory infiltrates, fibroblast activation, myocyte growth, nutrient flow, cellular metabolism and integrity of collagen matrix appear to have potential to modify healing and remodelling after infarction.

Diabetes mellitus and cardiac hypertrophy are two common pathological conditions which affect cardiac function and are vulnerable to ischemic injury. Feuvray presents myocardial metabolism as well as disturbances in membrane mechanisms which regulate intracellular pH in diabetic rats and their influence on the response to ischemic injury. Allard and Lopashuk discuss alterations in energy metabolism that occur in pressure overload-induced cardiac hypertrophy and how these changes increase susceptibility to ischemic injury. They also examine the relationship among altered energy substrate utilization, dysregulation of ionic homeostasis and functional recovery of the left ventricle. They suggest that glucose utilization may be useful target for pharmacological modulation in the hypertrophied heart.

On the whole, the book provides a rapid means to grasp the current level of understanding in a number of important areas related to myocardial ischemia. Many of the chapters are noteworthy for their detailed presentations, discussion of very recent developments and for providing future directions for investigations. An extensive and up-to-date reference list is available at the end of each article which facilitates search for the original studies. Each chapter is self contained and designed to be independent. Hence unavoidably, there are some areas of overlap in coverage. Some of these are cross-referenced by mentioning the author's name in brackets. Some of the chapters are crammed with information, that it will require substantial effort by readers with no background in pharmacology or physiology.

The book would have benefited from a chapter on pathological anatomy of myocardial ischemia and infarction with special emphasis on the effects of reperfusion. Similarly, a contribution from a practising cardiologist on clinico-pathologic correlates of acute ischemic heart disease syndromes would have made it more appealing.

The articles are not organized in the traditional format and the sections are uneven. The other deficiencies of the book are (1) the inconsistency in the format and style of various chapters (some of them have a summary, others do not. Most of the chapters have subtitles such as introduction and concluding remarks while these are absent in others), (2) an apparent over-emphasis on the possible therapeutic implication of each and every mechanism discussed, (3) an inadequacy of the index, and (4) occasional typographical errors.

There is no doubt that the book is an excellent addition to the available cardiology books. The extraordinary wealth of basic knowledge provided in the book could serve as a definitive reference to physiologists, pharmacologists, biochemists, pathologists and cardiologists interested in mechanisms of myocardial ischemic injury and treatment of related disorders.

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Indian Food: A Historical Companion. K. T. Achaya. Oxford University Press, Walton Street, Oxford OX2 6DP, UK. 1994. 325pp. Price: Rs 750.

There is an old story about an international group that was assigned the task of writing an essay on the subject of elephants. The Frenchman wrote on 'Love among the elephants'; the Jew wrote on 'Elephants and the Jewish question'; the American wrote 'Bigger and Better Elephants'; and the German scientist wrote 'An Introduction to the Bibliography of the elephant': Achaya's excellent book is not about elephants but it furnishes such a rich background in an area so close to our hearts that it appeals to all, to each in a different way. There is meat aplenty for the historian, main dishes for the social scientist and desert for the food scientist. Added to his previous detailed accounts of various other aspects of our food and culinary heritage he has given us, as one reviewer has noted. 'A superb repast'.

What makes a people unique and differentiates them from their fellow humans? Surely one of the most distinctive features of a culture is its food – that essential element in life that allows a diversity conditioned by climate, geography and human creativity. This book gives us appetizing glimpses into that hoary past and furnishes us a bridge between all the inventions, innovations and influences of our yesterdays that have made possible the wonderful specialities and daily fare of India today. Achaya, a distinguished oil chemist with a special bent for tradition and history has written with verve and interesting sidelights. Instead of assigning us to the sidelines, as mere consumers, he puts us in our rightful place as innovators and producers. This is an example of real 'people's science' and helps us to reaffirm our extraordinary capacity to create and evolve culturally and nutritionally balanced and delicious food, in harmony with the natural productivity of the earth. It puts us in touch with the wellsprings of our humanness.

We are now faced with a virtual assault on our traditional foods from TNC's and their universalized burgers, pizzas and fried chicken. Aside from the health issues involved, which arise from the overconsumption of too much salt and animal fat, we are forced to consider the political and economic issues of whether our limited natural resources and food grains should go to feed increasing numbers of battery raised fryers (chickens) or cattle for hamburgers rather than feed people. It takes 7–9 kg of feed to produce one kg of animal food. We are forced to consider whether we need 'ajinomoto' in our packaged foods or other chemicals, flavours and colours to ensure optical attraction and increase the shelf-life of packaged foods. We are forced to ask our government why we need Nestle Company to make our traditional pickles and savouries, why, indeed, we need any TNC in the food business in India! We are forced to ask even more importantly how it can allow others to patent our neem, our parboiled rice, extracts of our turmeric and any other foodstuff which will be mainly to their benefit, and little to ours.

We are also faced with the grim reality of more mouths to feed, less water to irrigate our fields, so-called structural adjustments to allow the market to dictate what should be grown and who can afford to eat it.

Written history ignores much of woman's part in humankind's progress. It undervalues her abilities and contributions as producer, inventor and processor. The arts of food processing and preservation as well as cooking have been mostly women's work. In many areas of the world, even basic cultivation, harvesting and storing of foods are still, today, in the hands of women. The essential quality of these inputs is obvious. It is too bad that few historians, including the one under discussion, have acknowledged this, but, hopefully, in an account like the present one, the real actors and actresses on the scene speak for themselves. In an urban setting, we tend to forget that milk comes out of a cow and tomatoes have to be planted, harvested and cooked before they become sauce. This book helps us put things afresh into proper perspective and appreciate the intricate hands on 'research' that has shaped our rich culinary heritage.

Achaya has taken us through both archaeological as well as written records, detailing the food of ordinary people and royalty, the influence of religious beliefs on menus and prohibitions, the vedic prescriptions for food as preventive medicine, description of royal feasts, accounts of what surely must be the original 'restaurant', the invention of ice creams and kulfi and descriptions of traditional utensils, and various apparatus used on both household as well as community scale in the preparation of food.

National cuisines and regional specialities are briefly described. We are sure the author himself would be the first to declare he has barely skimmed the surface of all there is to discover about the intracacies of such a vast subject but he has been able to mention some of the highlights of the various areas that will stimulate further research.

Chapters 14 and 15 catalogue the staple foods as well as some specified fruits, spices and vegetables with details of their origins and their adaptation into Indian cuisines. The last chapter lists imports from the Americas and other areas which are now firmly established as normal components of our menus. One of the most interesting facets of the story is the fact that the chilli pepper, an essential component of much Indian cooking, is imported from Central and South America. When and by whom is unknown but its Indian history is very ancient.

There are fascinating tidbits scattered throughout the book in separate boxes so as not to interfere with the text and to highlight related knowledge. There are abundant references, four very useful indexes, and many drawings, illustrations and coloured photos to round out the careful research and comfortable scholarship that is evident throughout. The original price of the hardbound book predicates a rather limited audience. A paperback edition would assure the much wider readership the book deserves.

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B. M. BIRLA SCIENCE PRIZES

The B. M. Birla Science Centre, Hyderabad, requests Heads of recognized Research Institutes and Universities for nominations for the B. M. Birla Science Prize in Mathematics, Physics, Biology and Chemistry for the year 1996.

The Prizes in each of the above subjects are for outstanding original contributions in the concerned field by young Indian scientists resident of India who are not more than 40 years of age on 31 December 1996.

All nominations should be sent in triplicate, including a full list of publications and up to five of the best papers as also the specific work for nomination, curriculum vitae and the formal nomination itself. These nominations should reach

Dr B. G. Sidharth
Director
B. M. Birla Science Centre
Adarsh Nagar
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before 15 August 1997.

All India Institute of Medical Sciences

Ansari Nagar, New Delhi 110 029

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Selected candidates will be informed latest by 30 July 1997 when the Course fee should be remitted. Details will be provided in the Selection Letter.

USING AGRICULTURAL DIVERSITY RESEARCH AWARD

THE AWARD

The Using Agricultural Diversity Award assists local groups and institutions in South Asia to undertake applied research on the use of agricultural diversity to meet the needs of farm households and protect the environment. The award is intended to encourage research collaboration, exchanges and dissemination of information on practical means to enhance the sustainable use of agricultural diversity by farm households. It recognizes the role that cultivated and non-cultivated agricultural diversity including livestock, play in the livelihood strategies and food security of rural people.

ELIGIBILITY FIELDS OF STUDY

Applications will be accepted for applied research on topics related to:

- Documentation of the varied uses of on-farm biodiversity in high biodiversity areas (e.g., pest control, drought tolerance, taste preferences, etc.);
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- Identification of socio-cultural factors influencing the use of diversity;
- Application of participatory approaches to plant breeding, management and use of biodiversity;
- Informing policy of the role of farmer participation in on-farm conservation and enhancement of agricultural diversity.

ELIGIBILITY

The award is targeted toward local organizations. However, it seeks to enhance collaboration between the informal and the formal sector (plant breeding institutions, national germplasm banks, etc.). Collaborative proposals are encouraged.

VALUE

The Award will cover justifiable applied research expenses to a maximum of 350,000 Indian Rupees. The award cannot be used for long-term salaries, institutional support or for the purchase of vehicles and other major fixed assets.

DEADLINES

Awards will be announced mid-June.

APPLICATIONS

The Award is governed by a diverse Steering Committee comprised of experts from national, regional and grassroots organizations in India, Nepal and Bangladesh as well as representatives from the international community. It is administered jointly by the Society for Research and Initiatives for Sustainable Technologies and Institutions (SRIST), Ahmedabad and the International Development Research Centre (IDRC, Canada).
Applicants should send a brief (3-4 page) description of the research topic to:

Prof. S. K. Sinha
Chair, Steering Committee
c/o International Development Research Centre (IDRC)
17 Jor Bagh, New Delhi, 110 003, India
Fax: 91-11-4622707
E-mail: UDRA@idrc.ca

The application should indicate:

- The Project title
- Name and full address of the principal researchers
- Description of the agricultural biodiversity in the region where the research is to be undertaken
- Research objectives and general workplan.

INDIAN SCIENCE CONGRESS ASSOCIATION

Announcement for Awards: 1997–98

(i) **Professor Hira Lal Chakravarty Awards:** Applications in prescribed forms are invited from Indian Scientists, below 40 years of age on 31 December 1996 with Ph D degree having significant contributions in any branch of Botany – either pure or applied. There are two awards, each carries a cash amount of Rs 4,000/- and a certificate. The awards are given on original independent published work carried out in India within three years prior to the award. Each awardee will be required to deliver a lecture on the topic of his/her specialization during the Annual Session of the Indian Science Congress in the section of Botany. Last date for submitting applications is 15 July 1997.

(ii) **Pran Vohra Award:** Applications in prescribed forms are invited from Indian Scientists below 35 years of age on 31 December 1996 with Ph D degree in Agricultural Sciences from any University or Institution in India, having made significant research contributions in any branch of Agricultural Sciences. Only research done in India will be considered for the award. The award carries a cash amount of Rs 5000/- and a certificate. The awardee will be required to deliver a lecture on the topic of his/her specialization during the Annual Session of the Indian Science Congress in the Section of Agricultural Sciences. Last date for submitting applications is 15 July 1997.

For proforma of application forms and necessary information, please write to the **General Secretary, Indian Science Congress Association, 14, Dr Biresh Guha Street, Calcutta 700 017.**

Department of Science & Technology
(International Division)

CALL FOR JOINT RESEARCH PROJECTS UNDER INDO-ISRAELI S&T COOPERATION

Department of Science & Technology, Government of India and Ministry of Science, Government of the State of Israel jointly invite applications from joint Indo-Israeli research teams for financial support under Indo-Israeli Agreement on Scientific and Technological Cooperation for developing and implementing research projects in specific areas of Biotechnology for crop improvement, Advanced Materials, Information Technology and Electro-optics. These projects may be planned for 2 years' duration and the financial support would be provided with an overall annual budget in the range of approximately Rs 10–15 lakhs/$ 30,000–40,000 for each side. The proposals are required to have the signatures of both Indian and Israeli coordinators.

Application forms along with guidelines may be obtained from:

Shri M. Bandyopadhyay, Director and Coordinator
Indo-Israeli S&T Cooperation
International Division
Department of Science and Technology
Technology Bhavan
New Mehrauli Road, New Delhi 110 016

Request for application form may be made latest by 10 July 1997 along with a self-addressed envelope (12 cm × 27 cm). Project proposals completed in all respects should be submitted latest by 24 July 1997.

Advt. also available on the Vigyan Prasar home page on the Internet at: http://vigyanprasar.com or http://www.vigyanprasar.com.

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Applications on plain paper in the following format with true copies of relevant certificates/testimonials should reach the **Chief Administrative Officer, NCRA–TIFR, Post Bag 3, Ganeshkhind P.O., Pune University Campus, Pune 411 007**, *on or before 10 June 1997.*

(1) Name of post; (2) Advt. No.; (3) Full name (underline surname); (4) Address for correspondence; (5) Permanent address; (6) Nationality; (7) Date of birth (attach certificate); (8) Names and address of two professors who can provide a confidential assessment of the candidate's academic performance, (9) Educational qualifications starting from SSC, with details of marks obtained; (10) Details of experience, if any; (11) Motivation for pursuing research in astronomy.

Candidates called for written test/interview will be paid IInd class (non a/c) train fare by the shortest route from their place of correspondence to Pune and back.

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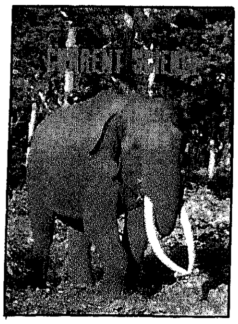

◀ COVER. A 25-year-old male Asian elephant with unusually long tusk for its age. (Photo: R. Sukumar). See page 885.

The editors thank Dr Santanu Bhattacharya, Department of Organic Chemistry, Indian Institute of Science, Bangalore for agreeing to be Guest Editor for the Special Section.

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In this issue

Tusk length, sex appeal and health status in elephants: The evolutionary logic of female choice

A fully grown Asian bull elephant with a magnificent pair of tusks is a truly impressive sight (as can be seen from the cover page of this issue). And not just human beings; female elephants too seem to find such tuskers very attractive – a state of affairs infinitely more important from the male elephant's point of view. Why should this be so? More generally, why do peahens prefer peacocks with longer plumage, why are stags with bigger antlers more popular (and so on. . .)? Even more generally, why do some qualities of the males make them more attractive to females?

Natural selection guides the trait of female choice (the way it guides everything else); any trait that leads to a production of better quality offspring is favoured, which, in turn, means that any trait which helps selecting a better quality (stronger, healthier) mate will be favoured. While body size is probably an obvious and a direct indicator of strength, secondary sexual characteristics such as bright plumage in birds have been speculated (and shown) to be reasonable indicators of health. In particular, in taxa as diverse as birds, fishes and reptiles, there is a strong negative correlation between the parasitic load (a possible indicator of health) and the quality of the secondary sexual characteristics; for example, male birds with bright, colourful feathers during breeding season have fewer parasites on them.

On **page 885** of this issue, Milind G. Watve and R. Sukumar report the extension of such studies to Asian elephants in the Mudumalai Wildlife Sanctuary. Using dung samples from individually identified elephants, they have been able to obtain an estimate of the number of intestinal parasites harboured by each of the elephants. They have then correlated this parasitic load with tusk length. A detailed analysis, involving data collected over five years from over 150 elephants shows that elephants with long tusks have lower levels of intestinal parastic infection. Female elephants thus do tend to choose healthier males, when they prefer mates with long tusks.

Unfortunately, such male elephants are even more eagerly sought after by ivory poachers! This has, in fact, lead to a rather severe depletion in the number of tuskers in that population. If the smaller parasitic load is because of genetic resistance, then, as Watve and Sukumar point out, poaching is selectively eliminating disease-resistant males as well. For a more detailed discussion of the long-term conservation implications (and also for several alternative explanations of why long tusks are favoured by females), see page 885.

N. V. Joshi

The chemistry–biology divide

Over three decades ago, C. P. Snow while delivering the Rede lectures at Cambridge coined the memorable phrase – 'The Two Cultures'. Snow was addressing the growing gulf between the sciences and the humanities. Over twenty years later, in an address to the American Association for the Advancement of Science (*Biochemistry*, 1987, **26**, 6888–6891), Arthur Kornberg borrowed the phrase to express concern about the increasing divide between the disciplines of chemistry and biology. In Kornberg's words – *'Chemical language has great aesthetic beauty and links the physical sciences to the biological sciences. Unfortunately the full use of this language to understand life processes is hindered by a gulf that separates chemistry from biology. The gulf is not nearly as wide as the one between the humanities and the sciences on which C. P. Snow focussed attention. Yet chemistry and biology are two distinctive cultures and the rift between them is serious, generally unappreciated and counterproductive.'*

In the decade since Kornberg's sharply worded essay, barriers between disciplines have become blurred. Even venerable chemistry departments have changed their names. For example, Harvard's Department of Chemistry has now been rechristened as the Department of Chemistry and Chemical Biology, a clear recognition that chemistry's most important challenges are derived from biology. The revolution in molecular biology has marginalized many classical disciplines under the broad area of biology. It has also profoundly altered the relationship between traditional chemistry and biochemistry. In Kornberg's words, – *'In its rapid and turbulent growth, molecular biology has washed away much of the bridge to chemistry. In the rush and excitement over the new mastery over DNA, attention in biochemistry departments has been sharply shifted to major biological problems of cell growth and development and away from chemistry. . . . Molecular biology falters when it ignores the chemistry of the products of the DNA blueprint – the enzymes and proteins and their products – the integrated machinery and framework of the cell. Molecular biology appears to have broken into the bank of cellular chemistry, but for lack of chemical tools and training, it is still fumbling to unlock the major vaults.'*

Conservative chemists have also jealously guarded their discipline and departments from 'biological contamination', thereby widening the growing gulf. Kornberg has a harsh assessment — *'The problem was that organic chemists placed arbitrary boundaries on their science. Although in their pursuit of natural products, they might still eagerly seek the challenge of an*

Amazonian butterfly pigment, they would not accept nucleic acids, proteins, and enzymes as proper natural products'. Kornberg is also unsparing in his criticism of others —*'Chemists were not alone in ignoring the chemistry of living things. The physicists who during the post World War II period turned their attention to biology, generally focussed on genetics and hoped to avoid chemistry for which they had little patience. Those who applied physical methods to determine the structure of proteins and nucleic acids expected that they could infer biological properties directly from the structures of these molecules and thereby bypass biochemistry. Many biologists were just as eager to remain clear of chemistry. Particularly so were those concerned with systematics evolution and behaviour.'*

Today the realization that biology abounds with immensely challenging chemical problems has largely demolished artificial barriers between the two disciplines, in the scientifically advanced countries. The growing appreciation that many practical and economically beneficial applications of science are facilitated by a fusion of disciplines has contributed in no small measure. Unfortunately, in India tradition is harder to break. Most departments of chemistry and biology (often further fragmented into botany and zoology) are yet to realize that puritanism is hardly a virtue in science. Universities set great store by the basic degrees possessed by prospective recruits to their faculties and pay little attention to promoting interdisciplinary interactions. It is therefore unsurprising that most students emerge poorly equipped intellectually, to comprehend the sweeping changes that are taking place at the interfaces of scientific disciplines.

This issue of *Current Science* presents a special section (**pages 788–845**) focussing on the chemistry–biology interface, as represented by a sampling of research from institutions across India. The articles touch diverse areas – bioinorganic chemistry, model DNA clearing agents, protein folding, antibacterial peptides, the immune response, time resolved fluorescence microscopy as a probe for intracellular dynamics – demonstrating that organic, inorganic and physical chemists have indeed begun to carve a niche for themselves in areas bordering biology.

Promoting research in interdisciplinary areas requires that collaborative interactions between scientists and institutions are facilitated. The tendency of evaluation committees to look askance at collaborative efforts, while assessing individual scientists must be banished. Even more importantly, adequate attention must be paid to the training of research students, such that overspecialization does not cripple them too early in their scientific careers. On this subject it is appropriate that Arthur Kornberg has the last word – *'In the long view, these cultural differences between chemistry and biology are dwarfed by our overall devotion to the larger culture of science. It is the discipline of science that enables ordinary people, whether chemists or biologists, to go about doing ordinary things, which, when assembled, reveal the extraordinary intricacies and awesome beauties of nature. Science not only permits them to contribute to grand enterprises but also offers them a changing and endless frontier for exploration.'*

P. Balaram

Land cover and land-use changes

Land cover and land-use changes have occurred in South and South-East Asia during the last century and earlier past of this century. The major driving forces for the same are population pressure, affluence and technology. The pattern of crops and their consumption have changed, leading to changes in groundwater depletion and pollution, and nutrient deficiency. There is a need to develop indices of unsustainability at the micro-level followed by regional cooperation as emphasized by S. K. Sinha (**page 846**).

T. N. Khoshoo

CURRENT SCIENCE

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CORRESPONDENCE

ISRO spy scandal

I read the correspondence entitled 'ISRO spy scandal – An unending witchhunt' (Dhawan, S. *et al.*, *Curr. Sci.*, 1997, **72**, 359). The charge against one of the ISRO's scientists S. Nambinarayanan was that 'he handed over drawings and documents relating to Viking engine and cryogenic technology to foreign/enemy countries in exchange for a large amount of money in US dollars'. The authors of the correspondence have given full details of the CBI findings and the verdict of the Chief Judicial Magistrate, Ernakulam which completely absolved Nambinarayanan of any wrong doing. They have concluded that 'there is no evidence to prove that espionage ever took place or that documents or money exchanged hands or that the accused including Mr S. Nambinarayanan benefited financially from these dealings'. If these statements are correct, it is really strange to see that Kerala High Court after going through all the evidence and statements on record has ordered further investigations in this case. After reading the order of the Kerala High Court, the authors have strongly argued the innocence of Nambinarayanan by saying that 'the clearance recently accorded by the Kerala High Court to further investigate the case will drag Nambinarayanan and his family through another period of misery'. I am also a space scientist and understand the whole case. Despite all the arguments placed by the authors, it is certain that the Kerala High Court Judge must have found enough reasonings in the CBI and Chief Judicial Magistrate, Ernakulam reports to order further enquiry. None of these reasonings are given by the authors except saying that further enquiry will drag Nambinarayanan and his family through another period of misery. In fact the basis for ordering further inquiry is the point of frontal attack. A good job done in elaborating this point could have provided relief to Nambinarayanan. As such the correspondence provides no relief to the incumbent. If the authors really wish to provide relief to Nambinarayanan, they should submit an *appeal* to the President of India questioning the basis on which Kerala High Court ordered further enquiry resulting into continued agony to Nambinarayanan. If favourable, the Presidential order on their appeal would constitute a meaningful relief to the sufferings of the incumbent. Writing one-sided and incomplete letters may not provide any relief to the incumbent and may even go against Nambinarayanan.

The authors have brought out some interesting facts which have been felt by many of the space scientists but it has not been stated in so many words. They say that 'like many large organizations, the Indian Space Research Organization tends to suffer from petty jealousies and professional rivalries that get translated into personal animosities'. In addition, ISRO may not be free from subjective personnel promotions and misappropriations in financial deals. The statement though made with a different objective and argument in mind, is literally correct for the present-day functioning of ISRO as a National Scientific Organization. I have had opportunities to work for and visit the NASA (USA) and ESA (European) organizations which very closely involve University's scientists and engineers. It is unfortunate to see that the space science in India though born on the University campuses has slowly drifted into the clutches of the Government Organizations. If this continues, within course of time, ISRO may become the epicentre of many more such cases. It is high time to review the functioning of ISRO objectively and ensure that it functions as an organization solely dedicated to the national cause.

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A scientist willingly working in strategic areas of research of a state or for profit-making activities of a company has inevitably to face associated professional hazards. No responsible government can afford to be complacent in safeguarding the national interest.

Unfortunately, the Second World War, and recently corporation-controlled trade wars have exposed the vulnerability of scientists to espionage issues. The espionage threat is integral to any secret research project, though sometimes honest scientists are tormented for no fault of their own. Robert Oppenheimer and David Bohm faced persecution, perhaps for political reasons.

I think the ISRO spy scandal should be viewed in this background. The most sensible approach open to an honest scientist is to get honourable acquittal through the court of law. Those who know that S. Nambinarayanan is not at all involved in this espionage case could provide him moral support, and help him to fight the case. Regrettably, the kind of intervention made in a recent letter (Dhawan, S. *et al.*, *Curr. Sci.*, 1997, **72**, 359) is likely to increase the problems of Nambinarayanan, and would harm

Indian science. The authors of the letter have been in close proximity to political power, and at the helm of affairs in the country. Surely they would not be unaware of the low credibility of the CBI, and yet they have quoted the CBI report as a gospel truth. The verdict given by the Chief Judicial Magistrate is mentioned approvingly. However, subsequent judicial action of the Kerala High Court is questioned. Will this help Nambinarayanan? The authors write: 'These dangerous trends, if allowed to continue, can demotivate and demoralize the many hard working and dedicated professionals who have made Indian achievements in these areas possible, usually for paltry compensations. Such actions are likely to derail these programmes and adversely affect the national interest more severely than any foreign hand'. What does 'paltry compensation' mean? Does patriotism need compensation? And, finally do we not find in the last sentence of the quote above an echo of the threat issued by the politicians against so-called judicial activism?

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Life in extreme environments

Although the earliest of life forms are believed to be of thermophilic origin[1], metabolism in most plants is adversely effected above 40°C. The lower optimal range for most species is influenced by earth's mean surface temperature, estimated[2] to be at 15°C. In higher plants, 10–35°C is optimal for photosynthesis[3], whereas extreme limits for active metabolism are around sub zero and up to 50°C in some alpine[4] and desert plants[5], respectively. 'Plant life under extreme environments' (H. Y. Mohan Ram and Promila Gupta, *Curr. Sci.*, 1997, **72**, 306–315) are the adaptive responses under these conditions.

However, the 3850 million years history of life[6] on this planet has evolved other glaring extremities, in the world of microbes. The development of succession flora in decomposing litter biomes is constantly modulated by the changing temperature regimes. The extreme and optimal temperature ranges are redefined each time one set of microbial population replaces the other. Sulphur oxidizing bacteria near hot water springs dwell at temperatures otherwise lethal for life. Infact, temperature as high as 80°C is the lower extreme limit below which certain archaeobacteria cannot survive[7].

Restriction of these organisms to specific niches may term rest of the environment as extreme! Perhaps not always. Crenarchaeota, an exclusive class of organisms, were long thought to be exclusively restricted to extreme environment of hot springs, but their recent discovery in marsh and lake sediments, and Antarctic ocean[8] has not only changed the existing concepts on microbial nomenclature but revealed greatest 'plasticity' that life can afford[9].

Life's endeavour to constantly proliferate in extreme of environments, goes a step further in the functional capacity of 'non-solar organisms', providing new hope of sustenance even when the lid of darkness may nudge down to seal life on earth[10].

Lastly, it would be of interest to look for organisms having a kind of facultative ability to shift from light to thermal-harvesting mechanisms, remembering that the first life forms used energy sources other than visible spectra of sunlight[1].

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Exodus of scientists from DRDO and ISRO

On 5 May 1997, in reply to a question put to the Minister of Defence in the Lok Sabha, the Minister of State in the Ministry of Defence, (N. V. N. Somu) revealed that 3 per cent of total strength of scientists/engineers resigned from DRDO in the last three years (131, 173 and 144 in the years 1994, 1995 and 1996 respectively.) He added: 'While scientists/engineers in DRDO have challenging tasks, certain percentage of them have joined multinational companies for better compensation (attractive salary and perks)', and 'suggestions have been made to Fifth Central Pay Commission and Empowered Committee of Secretaries for increase in the entry level pay and allowances for young scientists/engineers. In addition, improvements are being made in the provision of residential/hostel accommodation, medical facilities, posting in station of choice to the extent possible and enhanced training opportunities abroad.'

On 8 May 1997, in a reply to a question in the Rajya Sabha put to the Prime Minister on the brain drain from ISRO, the Minister of State, Yoginder K. Alagh, said: 'As many as 530 scientists/engineers have left during the last five years and personal reasons have been cited for such resignations. The liberalization of the economy since 1991–92 has provided them new employment opportunities in our country itself with high remuneration levels. The exact information in respect of persons who have gone abroad, is not available'; adding, 'The resignations of scientists/engineers have so far not had any serious impact on the schedule of the Space Programme. However, occasions did arise when schedule of critical work had to slow down for a while in a few areas owing to a number of scientists/engineers leaving almost at the same time'; and 'Better housing facilities, further opportunities for academic and related activities including further studies, enhancement of interface between scientists and industry are among the steps proposed'.

India accedes to Chemical Weapons Convention – National Authority established to oversee implementation

India is one of the original 130 signatories (State Parties) to the Chemical Weapons Convention (CWC). The negotiations on this treaty concluded on 14 January 1993 in Paris. The CWC came into force on 29 April 1997 after ratification by 65 nations. The Government of India has ratified the CWC.

The CWC prohibits the development, production, acquisition, transfer, use, and stockpiling of all chemical weapons by Member-States through a common regime that does not discriminate between the 'have' and 'have-not' nations. The CWC permits development, production, acquisition, transfer and use of toxic chemicals for non-prohibited use including industrial, agricultural, research, medical, pharmaceutical and peaceful purposes. Member-States are also under obligation to regularly and promptly report data on chemicals related to CWC to the International Secretariat through voluntary declarations, routine on-site inspections, as well as under 'challenge' inspections on demand, and after due-process.

To fulfil its obligations, each State Party has to designate or establish a National Authority to serve as the national focal point for effective liaison with the Organisation for Prohibition of the Chemical Weapons (OPCW) – a creature of the Convention – and with other State Parties to the Convention.

In order to fulfil India's obligations under the Convention, a National Authority has been set-up under the administrative control of the Cabinet Secretariat. The National Authority, besides serving as a national focal point for liaison with OPCW and other State Parties, will also be responsible for the collection of data, fulfilling of declaration obligations, negotiating facility agreements, coordinating OPCW inspections, providing appropriate facilities for training national inspectors and industry personnel, ensuring protection of confidential business information, checking declarations for consistency, accuracy and completeness, registration of entities engaged in activities related to CWC etc. For carrying out its functions, the National Authority will be suitably empowered by enacting legislation to be introduced by the Government of India.

The National Authority, which started functioning from 5 May 1997, is headed by a Chairman, who will be supported by a suitable Technical Secretariat to look after the various functions. A High Level Steering Committee under the Chairmanship of Cabinet Secretary with Secretary (Chemicals and Petrochemicals), Foreign Secretary, Secretary, Defence Research & Development, Defence Secretary and Chairman, National Authority as its other members, will oversee the functions of the National Authority.

A promising hybrid vaccine against malignant tertian malaria

C. C. Kartha

A group of scientists from SmithKline Beecham Biologicals, Reinsart, Belgium; Water Reed Army Institute of Research, Washington DC and Naval Medical Research Institute, Bethesda, Maryland have developed a recombinant anti-malarial vaccine that incorporates adjuvants to enhance the immune response. Stoute and colleagues report in *The New England Journal of Medicine* that the vaccine protected most of the vaccinated volunteers against infection with *Plasmodium falciparum*, when they were later exposed to *Anopheles* mosquitoes infected with the malarial parasites[1]. Their results raise our expectations for success in the quest to develop a vaccine against malaria.

P. falciparum, the infectious agent of malignant tertian malaria, a serious form of the disease, has a complicated life cycle. Vaccines have been designed targeted at different stages of development of the parasite[2]. The first infective stage of the parasite is the sporozoite. Sporozoites enter the human blood through bites by infected mosquitoes. Blood carries them to the liver where they invade the liver cells and multiply. Tens of thousands of merozoites are thus produced and large numbers of them are released from the liver cells. The merozoites enter the blood stream again, invade the red blood cells in which they further undergo transformation and multiplication. It is this phase which is responsible for the symptoms of malaria. Some of the parasites in the red blood cells metamorphose into gametocytes which differentiate and multiply, resulting in sporozoites which resettle in the salivary gland of mosquitoes.

A number of stage-specific antigens have been identified as potential targets for vaccine development. Several candidate vaccines have been evaluated both in experimental animals and human volunteers[3].

Initially, irradiated sporozoites were tested in volunteers and were found to induce both humoral and cell-mediated immunity[4]. The method consisted of exposing individuals to repeated bites of a large number of irradiated mosquitoes infected with *P. falciparum*. The large dose required (400–1800 mosquito bites per person) made this approach practically disadvantageous. A decade ago, the gene for a protein expressed on the surface of the sporozoite and its stage in the liver cells, was cloned and structure of the protein identified. This circumsporozoite protein (CSP) was demonstrated to have a role in protective immunity in rodent models of malaria. Synthetic peptides corresponding to the immunodominant epitope of the CSP, conjugated to tetanus toxoid as a carrier, have been tried as vaccines in clinical trials, but were found to have negligible protective efficiency. Another vaccine which consisted of a recombinant protein made from the CSP and conjugated to *Pseudomonas aeroginosa* toxin A was found to be safe, well tolerated and immunogenic. However, the immune response was seen to be short lived in a clinical trial in Thai soldiers[5]. The problem with sporozoite vaccines is that the vaccine will fail if a single sporozoite escapes immune suppression.

Several vaccines against merozoites have been developed. They are not protective against infection, but may diminish tissue damage and thus lessen morbidity and mortality. Among the merozoite vaccines, a synthetic Spf66 vaccine developed by Patarroyo and colleagues in Columbia gained considerable attention and was reported to be efficacious[6]. The vaccine is a combination that incorporates three merozoite peptides and CSP. Field trials using the vaccine were conducted in Tanzania, Columbia, Papua New Guinea and Ecuador, regions endemic for malaria. The protective efficacy of this vaccine as reported by various investigators range from 3% to 89%. When one considers that protective efficacy against natural infection itself is about 60–80%, the recent reports that the protective efficacy of the vaccine is only 31% in children in Tanzania and 8% in infants in the Gambia are not encouraging[7,8]. A randomized double blind placebo-controlled trial in children of north western Thailand also did not confirm the usefulness of the Spf66 vaccine[9].

Yet another strategy for vaccine development has been to prevent malarial gamete formation or maturation of zygotes in an attempt to block transmission of infection to mosquitoes. Since the antigens seen in the sexual stage of the parasite are not expressed in the stages that affect humans, the parasite may not develop resistance to transmission-blocking vaccines.

According to estimates of the World Health Organisation, 500 million clinical cases of malaria are reported annually and malaria causes up to 2.7 million deaths per year. Rapid spread of drug resistance in the parasite and evolution of *Anopheles* mosquitoes unsusceptible to insecticides are obstacles to containment of malaria. Vaccines appear to be a likely means for effective control of the disease, though an ideal vaccine is still elusive. It is in this context that the RTS, S vaccine has emerged to provide cheer.

The hybrid vaccine, developed by Stoute and co-workers, has a single polypeptide chain corresponding to amino acids 207 to 395 of *P. falciparum* (3D7 parasite) fused to hepatitis B surface antigen (adw Serotype) and a polypeptide of 226 amino acids corresponding to hepatitis B surface antigen. These polypeptides spontaneously form composite particulate structures during their simultaneous synthesis in yeast. The vaccine was earlier shown to be significantly more potent than previous CSP formulations[10]. The present study evaluated three formulations of the hybrid vaccine. Vaccine 1 contained RTS, S, alum and an immunostimulant monophosphoryl lipid A. Vaccine 2 consisted of RTS, S in an oil–water emulsion. Vaccine 3 had RTS, S in oil–water emulsion plus monophosphoryl lipid A and an adjuvant QS 21.

Forty-six individuals (18 to 45 years of age) who were previously not exposed to malaria were given the vaccine. Fourteen subjects received vaccine 1, fifteen received vaccine 2 and vaccine 3 was administered to 17 persons. Twenty-seven subjects were given 3 doses and others received 2 doses. Approximately three weeks after the last dose, they were subjected to sporozoite challenge until 5 infected mosquitoes had fed successfully. All individuals were followed up monthly thereafter and those who remained asymptomatic and had no parasites in their peripheral blood for 60 days after challenge were considered to be protected against malaria.

All persons who received 2 or more doses of the vaccine developed antibodies against CSP. Antibody levels were found to peak after the second dose, then declined between second and third dose and latter attained maximum levels after the third dose. There was considerable variability in antibody response among individuals and also to different formulations of the vaccine. Responses to vaccines 2 and 3 were significantly greater than those to vaccine 1. Among those who completed the study, only one of seven subjects given vaccine 3 became infected, while 7 of 8 given vaccine 1 and 5 of 7 given vaccine 2 developed malaria. Parasitemia was found in all the six subjects, who were not immunized and served as controls, 11–13 days after sporozoite challenge. The vaccine efficacy was estimated to be 86% and relative risk of infection was computed as 0.15. Protected individuals had higher antibody titres.

In their earlier studies the investigators had demonstrated that vaccine 3 is superior for inducing strong antibody responses and antigen specific delayed hypersensitivity in primates. In mice, vaccine 3 was found to stimulate proliferative and cytolytic T cell responses as well.

A remarkable feature of the RTS, S vaccine is the conspicuous effect of certain adjuvants on the vaccine efficacy. This observation suggests that immune potentiators capable of upregulating protective cytokine production or leading to advantageous immune cell responses may be combined with anti-parasitic elements in developing more potent vaccines[11].

The crucial test for the RTS, S vaccine is yet to be done. A randomized double blind placebo-controlled field trial in areas of malarial endemicity would determine whether the hybrid vaccine has an edge over the other vaccines on trial.

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OPINION

Experimental statistics for biology students: An experiment in motivation

V. Sitaramam

A major fallacy exists in teaching that the mere existence of a subject necessarily motivates the students to go after it, merely based on teachers' advice. Turning a blind eye to what is not immediately necessary is called short-sightedness in students and dedication to specialization and pursuit of depth among the faculty. Thus, biologists end up learning mathematics and statistics for reasons of 'subject' which necessarily leads to third rate statistics and even worse mathematics (note 1). These problems worried us at the Biotechnology programme at Pune University since we take students with both biology and quantitative backgrounds. A sustained dialogue over years with freshly admitted students in basic sciences such as zoology and biochemistry and the better-funded courses such as the National Biotechnology Masters programme wherein biochemistry is an important component, as well as with graduate students enrolled for Ph D in these sciences at the University of Pune, revealed that, with the exception of agricultural students, any background in statistics is generally non-existent. Background in mathematics is also very poor among students of biology and even in general chemistry. The practical, laboratory training that they have had is also non-existent. Mensuration, taught to engineering students routinely, is non-existent in all sciences at the bachelor level (note 2). Even the idea of a derivative requires to be painfully taught to students who are already past 20 years of age. The major problem is that they are not motivated since they see no particular point in learning it. Increasing emphasis on cookbook (qualitative) procedures, kits and readymade reagents have rendered quantitation and kinetics of any kind in subsequent years increasingly extinct (note 3).

The biotechnology programme at Pune University has been documented at length with regard to its components (see refs 1–3) and represents one of the few University programmes in India in which the progress (note 4) has been kept track of by continuously monitoring various aspects of student performance (note 5). The existential concerns related to the need to emphasize the quantitative aspects of biology (note 6) have motivated us in the teaching programme to introduce interactive experiments that convince students that there is more to science than what meets the eye. The task is three-fold; firstly, the experiments should be posed as statements simple and commonplace enough to be understood, and yet complex enough to be nearly unanswerable; secondly, students should understand that facts do not speak for themselves and are to be made to speak; and thirdly, computers and computations, statistical and mathematical, actually make the tasks easier and not obtuse and difficult. The last is of particular importance since the opinion of students on the available books varies from 'boring' to 'forbidding rather than friendly'.

A sustained effort was made to shake up students from a rather indifferent if not negative attitude towards all things quantitative (ref. 1). We need deceptively simple experiments that actually make the students *wary* of their own observations and conclusions (note 7). This is aimed at achieving specific dividends: firstly, the message could be made clear that, measurement is a matter of serious concern, leading to increased interest in practical training; learning statistics and mathematics could be of advantage, improving the attendance and attentiveness in these subjects; and lastly, students grasp the need to ask and answer aggressively what a method or procedure means and that this question would be meaningless without verification or testing. I outline here a couple of introductory experiments on measurement, introduced at the beginning of a course in physical biochemistry and conducted over the last eight consecutive years (note 8). The experience may be summarized as a worthwhile effort (note 9), primarily based on student feedback.

Problem 1

On day one, the first year students are assembled with the following question:

'You students are admitted *via* an entrance test at the national level. By some strange machinations in New Delhi, we got wind of fact that, this year, the selection was highly arbitrary (note 10). The selection was exclusively based on body size: only the shortest of the girls and the tallest of the boys have been chosen. Prove or disprove.' They are asked to freely discuss among themselves during lunch and come back with a procedure to be implemented the same afternoon. They are forbidden to discuss this problem with the seniors in the second year, for which the seniors also fully co-operated. There are about 18–20 students per year.

What I describe here is a set of common responses and how the experiment proceeds. No two batches respond the same way, making the experiment always interesting to the teacher. It is also amazing how often the students attempt to describe the solution free of all jargon (which they are yet to learn) and how the basic idea is understood by nearly all intuitively. Some working knowledge in statistics for the instructor helps. It is important not to help the students.

The solutions

The students take the problem initially as a joke and at least 20% claim that they can settle it in an hour. They state that they wish to measure and ask for an expandable scale of the kind one finds in a clinic. Since the department has none, they settle for a tape and use the wall to mark the heights and measure these heights with a tape. Less than 10% of the students explicitly realize that one needs some device that projects perpendicularly from the wall to mark the height while avoiding error due to parallax. They are all familiar with the idea of the least count. They are emphatic that the error for measurements with a metric tape would not exceed the least count of 1.0 mm. They accept that some of them are good in measuring and some may not be and that it is worthwhile to insist that everybody measures everybody else and that we can also figure out how good the capability is. They do not speculate how to go from these measurements to the actual question. They reduce the problem to what they can handle first: Are the girls taller or shorter than the boys? They know that they should compare the mean heights. In another two hours or so, they prepare 19×19 matrix (note 11). A table is made and they obtain the mean values as well as ranges of heights. They can see that given a person x, his/her height taken by everybody has less variation, i.e. lower range than the whole class. Surprisingly this range is larger than 1.0 mm and usually goes up to 3–4 mm, i.e. 169.3–169.7 cm for the height of x; $n = 19$. The students are asked to make up their mind: was that a good measurement? The answer at that point has always been negative. The measurement would *never* match their initial notions of perfection. From this point onwards, a number of conflicting hypotheses are thrown at the group by the teacher. The first suggestion is that the tape is faulty either in the markings being non-uniform or that it is made of rubber and tends to be elastic and varies depending on the pull.

The students deny the first and swear that the second cannot be true, since they have taken a metal tape and that the room did not get appreciably hotter during the measurement. They begin to enumerate where each could have gone wrong. Some of them could be poor in handling the measurements. How can they find out? The problem is that the lowest error as detected by the range is still 2–3 times the least count. It is so regardless of whether the persons, the measurer or the measured, are tall or short. The range dramatically comes very close to the least count when the scoring of the height marked by one student was visible for the next person while marking on the wall. The students agree that it is a bad procedure since one is not really measuring and is only trying to conform to the previous measurement. They intuitively agree that the 'blind' measurements are objective and superior to such biased measurements.

At the second stage, the students compare the heights of all individuals in the class and then as sub-groups of boys and girls. Now the range is much larger from the tallest to the shortest. They did not have difficulty in understanding why the range should be very large, as much as 32 cm. The notion of biological variation among individuals is something they understand very readily. How this can be proven to exist is another matter since measurements themselves have variation! When they are asked the question whether this difference was due to error in measurement, the response was immediate, No. The reasoning, however, was slow to come. They are introduced to the terminology of intra-individual variation and inter-individual variation. It was suggested to them that it would perhaps be all right if the inter-individual variation was much larger than intra-individual variation to believe a measurement (note 12). Thus, if boys are taller than girls, this statement is reliable if and only if we provide a view that the inter-individual variation is much larger than intra-individual variation within multiple measurements, least count representing the lowest error that could be possibly recorded.

At each stage they are egged on to answer the starting question. They feel strongly that boys being taller than girls could be universal which may have nothing to do with the selection process. In fact, all of them feel that such a statement is not possible unless we have the heights of all the students who appeared for the examination including those who have passed.

Then the students are introduced to the calculation of standard deviation and standard error of mean. There is no difficulty in understanding the meaning of mean; standard deviation takes a little more time. It is suggested to them that the range would be roughly four times the standard deviation. This leads to a discussion of frequency distribution, and the meaning of 95% confidence limit could be appreciated. They definitely like the idea that an error follows some rules in real measurements. The difference between a systematic error and random error becomes self-evident and there is no real difficulty in appreciating that even random errors follow some rules. Standard error of mean takes even a longer time to comprehend. The fact that each individual measurement as well as the mean itself could vary is understood; then standard error is also understood. It is also easier to understand that the standard deviation would be larger than the standard error of mean. (They are amused by the suggestion that one prefers to illustrate in publications data with standard error than with standard deviation!) The equations corroborated the view that mean would tend to vary less than the individual measurements. Measurements as duplicates, triplicates and so on are appreciated as a good strategy to enhance the reliability of measurement.

By this time (usually 2–3 hours each of two sessions at least were spent by then), the students agree that there is nothing called a 'perfect' measurement and also that how good a measurement is can be quantitated. Calculations by hand or with pocket calculators were painful for those who did not know how to use a spread sheet on a computer. They all agree that computers are definitely worthwhile if the data analysis is to be kept to a fraction of time for data acquisition. By then, the polarity in the class becomes very strong. The vocal invariably take the position that the question is not answerable with the data available. There are always one or two doubting Thomases. One went to the departmental office one year and obtained the marks/ranks obtained during selection in Delhi and showed that there was no difference between the means and standard deviations of performance for boys and girls, nationally and those for Pune alone, though their heights at Pune differed. In a span of eight years since these practicals were started, not even once did the students give a suggestion of a procedure. Once a student did argue that there were more boys above the mean value for themselves while the girls did not reflect this. There was massive confusion how more number of boys could be above their own mean.

Then the idea of a frequency distribution is introduced and discussed by the instructor with emphasis on the nature and meaning of skewness. Symmetric and asymmetric distributions are identified. The students immediately plot the frequency distributions from the data and look for skewness. The problem is translated into Figure 1 and then the students agree that there could be such a possibility. They also are of the firm opinion that this does not prove the point but merely affirms the possibility. At this point they are introduced to the idea of a *t*-test. They use this to check that boys and girls do differ in their mean heights; then they compute skewness and kurtosis. The students begin to sense that effective comparisons are meaningful only if the curves of frequency distributions look nearly the same. Various Pearson distributions, when formally illustrated, amplify this feeling. They all agree that it is desirable to have same number of observations in the groups for an effective comparison, considering the difficulty in getting good frequency distributions at smaller numbers. They simultaneously learnt the art of dividing observations into class intervals and about uni- and multi-modal distributions. For this, they combine the data of boys and girls and see whether the frequency distributions tell them if they are subgroups. They understand that existence of a single apparent uni-modal distribution need not rule out subpopulations, in as much as poor selection of class intervals could make every group a subpopulation! They see that there is a role for a good interpreter and that the use of statistics does not preclude the use of human mind and that it actually augments it. That is considerable progress since the best they could come up with before the experiment was something like lies, damn lies and statistics.

Usually there are some adventurous souls who try to make something like paired data out of boys and girls and fail miserably. Failure of an idea is more welcome than having no idea at all. We generally found it necessary to see to it that the students exhaust all possibilities that they could imagine. Testing has no

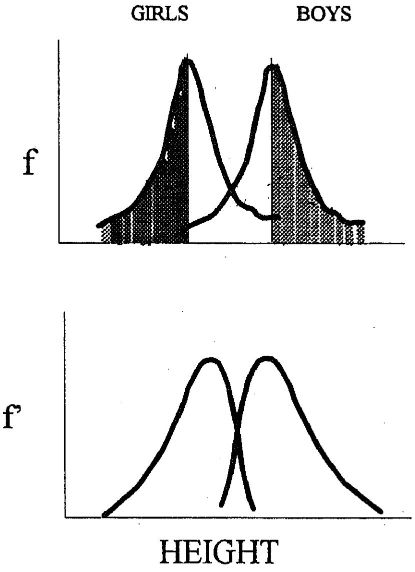

Figure 1. Problem 1 is depicted pictorially. Top panel shows that the frequency distribution of boys and girls is different. The mean/median/mode of this symmetric distribution is indicated by a vertical line for each distribution. The hypothesis implies that only the shaded lot are selected: the taller among the boys and the shorter among the girls. The bottom panel spells out the kind of distribution created by the suggested selection bias: note the asymmetry (skewness) of the distributions in the bottom panel and how it differs for the boys and the girls.

The discerning reader (to date only a referee and not even one student) would note that *f'* does not follow from *f*, given the rule as stated in the top figure by deleting the non-shaded area. The rule needs to be modified. The answer is simple but will be left unanswered here. Incidentally an important component of this kind of instruction is that answers need to be found by modelling.

meaning if alternatives are not imagined. It is necessary to play on every spontaneous idea and create a measurement around it so that the ideational process gets aroused and encouraged. By now the students heartily agree that measurement is the right thing. Subjective ideas are no good. It is possible to veer the discussions towards realistic situations wherein the importance shifts to paired data.

There were years when the data suggested that the starting premise was right and there were years when the starting hypothesis was rejected outright. The former was most instructive in understanding what a chance association is. The students actually take 20–30 sets, each of 8–10 random numbers, and do *t* test to see if the significance ($P < 0.05$) ever emerges due to chance alone. Since it always does, such results have a chastening effect on their thinking.

Then the second problem is posed to them.

The second problem

It is not possible to accurately transfer sugar into a beaker just with a spoon and without a balance. The initial reaction is affirmative. The test procedure is also conjured up readily. Take one spoonful, two and three and so on and see how reproducible the weighment per spoon is. When they do the experiment, they are asked to test how good it is. A few promptly convert it into 4–5 measurements of weight per spoon, though they initially plot a linear relationship between number of spoons of sugar and the weight. They get the mean and standard deviation. However, they are at a loss to assess just how good the spooning is!

They are introduced to linear regression by the method of least squares (note 13). They assess the slope and intercept as well as the coefficient of correlation for the X on Y as well as Y on X regressions. Linear regression by the method of least squares has rather rigorous assumptions which are worth knowing. Foremost of them is the point that one has to choose the variables correctly for the axes. The method assumes that the error is in assessing Y such as the height of the individual (or the absorbance of a reaction) and the age (or the concentration of the substance like glucose) which is generally, and these days, reasonably known. They are startled to see that the slope and intercept are not the same while the coefficient of correlation remains the same when they do linear regression after transposing the axes one for the other. After the exercise, they understand readily the three cardinal assumptions of linear regression, of the error being on Y, normally distributed, and constant at all values of X. This last assumption, viz. homoscedasticity, becomes not too difficult a concept after the experiment. They guess that in real measurements, error would be high at low values measured since they approach the least count. They are already familiar that actual error due to intra-individual variation would be larger than the least count! They agree that homoscedasticity is a pipe dream in difficult measurements, i.e. at the limits of sensitivity.

More striking is the realization an year later that biochemists generally teach the plotting of standard curves the wrong way. There were at least half a dozen students who argued in these eight or more years that, to assess the unknown protein or glucose, they should regress the reverse way, i.e. X on Y and not Y (the colour) on X (the glucose or protein concentration) for the standard curve. There have been some embarrassing moments due to conflicts with teachers who would usually insist on Y on X regression. A more common response from teachers (even from some of the referees of prestigious journals, in my experience) is that it does not matter if the assays are of 'quality'.

The next level becomes really interesting when there is an opportunity to discuss with the students that what is important is the variance and not the mean. An explanation of $(1 - r^2)$ being the variance unaccounted for by glucose and this should be the lowest for a good measurement brings home the all important message of quality control. There is a scramble for who gets the lowest $(1 - r^2)$ in the spoon experiment. At least one or two repeat the experiment just to prove the instructor wrong that nearly perfect fits can be obtained by measuring by eye alone. Bless them.

Concluding remarks

The distractions are the most important part of this instructional process. The students do not mind being proved wrong as long as this is for a good reason. Every group has its own leaders. It is fascinating how these self-styled leaders get cut to size by their own inflexible positions in such group interactions. This has the salutary effect of promoting intellectual anarchy or free thinking. These are not just *post facto* analyses by the author. Students take part in the subsequent months in such discussions and laugh about it. Among the major benefits from this experiment are the ease with which chromatography (wherein the width of the eluting band has interesting possibilities on the source of error) could be taught later, as also a sensitization to non-normal errors as in radio-immuno-assays. Kinetics could be taught more ambitiously and rather critically (note 14). These exercises are not without drawbacks. Major among them is what the instructor has to face. After one semester of such a set of experiments, he never gets an immediate reply for any question from any student than 'it depends'! They become professorial, leaving the instructor to make all the mistakes.

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Notes

1. I refer to the majority rather than the gifted minority since this minority does not need these views.
2. We must not consider medical students among these since the Indian medical student learns any and everything that he can only by rote. Most of the modern biology that these Western medical textbooks increasingly refer to remains as strange as voodoo in most medical colleges where science must be taught by those who have medical and not science degrees.
3. It would be disconcerting if the future generations of biochemistry students find it difficult to derive even the km.
4. As well as the lack of it.
5. As also the departmental, by national committees over the years.
6. A concern shared only with the IITs and JNU, Delhi, while we at Pune remain the only University with unrestricted admittance from either stream.
7. In fact, this is the major bane of teaching in biology wherein hypotheses are preferred

to be accepted unquestioningly rather than be understood for their internal contradictions and uncertainties. Teaching takes the same dogmatic route.

8. A subdued version of this same experiment may be carried out using high school students of 14–16 years age without a significant loss of content.
9. This is in spite of the fact that the experiment does involve a lot of effort and tends to be viewed not as biochemistry/biology experiment by fellow teachers. I am yet to come across a statistician who believes that the experimental component of biology is any of their concern in teaching.
10. A disclaimer is perhaps now in order that the problem does not refer to any actual instances and any resemblance to reality is purely a coincidence.
11. Following the well-known aphorism 'a barber is one who shaves every one except himself'.
12. It often comes as a revelation that there is no absolute measurement and even that a measurement need not be far better than what the conclusions warrant.
13. Often one needs to estimate the concentration of protein/glucose in an unknown sample. The standard way to do it is to prepare solutions with known concentration, and measure some property, which is usually the absorbance. The plot of absorbance (Y axis) vs concentration (X axis) is known as the standard curve.
14. It is interesting that the author never came across a paper that critically tested whether the point of intersection of the reciprocal plots to detect the order of the reaction was actually above or below the line ($1/v = 0$). It is the faith and not the substrate that can reach a concentration of infinity readily.
15. Throughout the text, I deliberately omitted commonplace statistical definitions and equations to emphasize the need for intuitive understanding. Nearly everything here and much more is available in any primer on statistical methodology, except the motivation to open these pages.

ACKNOWLEDGEMENTS. The Biotechnology programme at Pune is supported by the Department of Biotechnology, Government of India. Presence of a good statistics department within a stone's throw on the campus proved to be mutually beneficial over the years in both the teaching programmes and research: we obtained the procedures and they, the calculi. This paper was prepared during the sabbatical year of the author at Rio de Janeiro, supported by a grant (#300917/95-3) from Brazilian National Research Council – CNPq – towards a visiting professorship. Those desirous of more details of the procedures and extending these to routine biochemical experiments may contact the author at Pune.

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SCIENTIFIC CORRESPONDENCE

Experimental annual forecast of all-India mean summer monsoon rainfall for 1997 using a neural network model

Accurate long-range forecast of rainfall can help in improved planning of agricultural strategies as well as in crisis (e.g. drought) preparedness. However, conventional methods for such forecasts, based on either statistical or dynamical methods, have limited skill in long-range prediction of monsoon rainfall. This is particularly true for prediction for longer than a season in advance. Although some of the statistical models have shown considerable skill in forecasting Indian summer monsoon rainfall (ISMR)[1], their range does not exceed a few months. Besides, most of these statistical (regression) models require a number of observed parameters, many of which are not available until before onset. This has been one of the major obstacles in attempts to generate very long range (VLR, one year or more) forecasts of monsoon rainfall.

In an attempt to develop an alternative successful methodology for generating VLR forecast of ISMR, the technique of neural networks was explored[2]. The usefulness of neural networks for simulating atmospheric process has been emphasized in recent years[3]. However, extensive experimentation and evaluation revealed the conventional neural networks to be inefficient to generate VLR forecasts of ISMR with skill and consistency. However, it was shown in a recent work that a generalization of the structure of a conventional neural networks, termed cognitive network (CN), has the potential for generating VLR forecast of ISMR with good accuracy[2]. Subsequently an extensive statistical evaluation of the performance of CN was carried out by generating 73 hindsets for the period 1821 to 1993 for different test cases. Results of these analyses, presented elsewhere[4], show that the CN can generate annual forecast with average (over 73 cases) error less than half the standard deviation of the data, with absolute error about 36 mm. Encouraged by these results, an experimental annual forecast of ISMR was generated for 1996 using this method as 866 mm.

These forecasts were generated using ISMR data for 50 years prior to 1995 taken from Parthasarathy *et al.*[6]. The forecast for 1996 was 866 mm, which is about 102% of the mean of the 120-year data used in our study. The corresponding observed value is also 102%, indicating an excellent agreement of our experimental forecast with observation. However, it should be mentioned that the (yearly) normal used by the India Meteorological Department is somewhat different from the simple 120-year mean considered by us[7]. The purpose of this note is to record experimental forecasts generated by us for 1997 and 1998, as 846 and 945 mm respectively.

The purpose for generating forecasts for 1997 and 1998 is that it will contribute to an objective evaluation of both the skill and the range of the forecasts.

It should be mentioned that both the forecasts for 1997 and 1998 (and previously for 1996 as well) were generated as an average of an ensemble of forecasts for the respective year. These ensembles were computed by generating a large

Table 1. Experimental annual forecasts for 1997 and 1998 from ensemble forecasts

Configuration	Iteration in 1000	Forecast rainfall in mm 1997	Forecast rainfall in mm 1998
	5	860	946
	10	862	949
	15	861	947
A	20	860	944
	25	863	942
	30	864	943
	35	863	945
Mean (mm)		862	945
Standard deviation (mm)		1.5	2.8
	5	863	946
	10	865	949
	15	859	948
B	20	860	941
	25	863	943
	30	866	945
	35	864	939
Mean (mm)		863	944
Standard deviation (mm)		2.4	3.4
	5	860	944
	10	863	940
	15	864	945
C	20	865	949
	25	865	946
	30	866	943
	35	865	948
Mean (mm)		864	945
Standard deviation (mm)		1.9	2.8
Final mean (mm)		863	945
Final standard deviation (mm)		2.1	2.8

number of forecasts for each year by varying the number of iterations. The network configuration, as well as other network parameters were identical to those used for statistical evaluation of the network for 73 hindcasts. The range of iteration itself was determined as that which provided the best result (minimum absolute error) in case of the 73 hindcasts. For clarity of presentation and to indicate the dispersion (standard deviation) of the forecast with the number of iterations, we present in Table 1 the mean and the standard deviation of the ensemble forecast, for three network configurations. The experimental forecasts quoted here are the final mean of all these ensemble forecasts. The (mean) standard deviation of the forecasts thus provides a measure of precision of our forecasts.

Once again we want to emphasize that this forecast is a purely experimental one, to provide an objective evaluation of the forecast skill of our method; it is thus not meant for any operational use. A general weakness of the method is that it often fails to capture very large (more than twice the standard deviation) departures from the mean, a weakness shared by most statistical methods. It should be noted that 1997 is also predicted to have a normal monsoon, which will be the tenth consecutive normal monsoon. In contrast, 1998 is predicted to have an excess monsoon. It will be interesting to check next year how our forecast compares with observations.

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Temporal variation in gravity field during solar eclipse on 24 October 1995

Solar eclipses provide an unique opportunity for the study of celestial phenomena such as different parts of the sun like corona and chromosphere, its atmosphere and their interaction with the earth and its atmosphere. The solar eclipse on 24 October 1995 starting from sunrise at Iran and ending at sunset at the Pacific Ocean provided a 46 km wide strip for approximately 1800 km in India from Neem Ka Thana (Western Rajasthan) to Diamond Harbour (West Bengal) where total solar eclipse was observed for some time between 7.22 am to 10.30 am (ref. 1). This solar eclipse was unique in several ways due to social consciousness and several scientific experiments were conducted which provided several interesting results[2].

During this period of solar eclipse, we happened to be at Dhoraji (21°44′; 70°27′;

Saurashtra) in connection with the gravity survey for oil exploration in that region. This region falls in approximately 80% of the total eclipse and we recorded the temporal variation in the gravity field at this place continuously for approximately 12 h before and after the eclipse. The variation in the gravity field is recorded using a Lacoste–Romberg gravimeter of 0.01 μgal accuracy. The temporal variation in the gravity field recorded at a station can be broadly classified as: (1) Very large period (10^2–10^4 years) variations related to the mantle processes, sea level changes, glacial rebound and ice processes. (2) Large period (10–10^2 years) variations due to core–mantle interaction, plate boundary deformation, etc. (3) Medium period (days to years) variations due to earthquakes, volcanoes, etc. (4) Short period (hours to days) variations caused by drift of the gravimeter, ocean tides. (5) Shorter period (hours) variations due to the sudden changes in the atmosphere such as pressure and temperature. (6) Shortest period (seconds to minutes) high frequency noises which are sharp and sudden.

As mentioned above, these variations are largely characterized by their wavelength which is used to classify them in different groups. In the 12-hour record which we made, we can neither record nor identify the variations classified under groups (1), (2) and (3). The fourth group of variations show a cycle of 24 h and is represented by smooth cyclic changes as given in Figure 1 which represents the tidal variation on 24 October 1995 at Dhoraji. It is the group (5) kind of variations which can be recorded in our gravimeter and are of present interest. The variations under group (6) are easily identifiable as they are sharp changes due to sudden jerk or some motion in the vicinity. Some experiments of recording gravitational field during solar eclipse[3,4] were conducted previously to find out the tidal effects during that period and gravitational shielding (Majoranna effect).

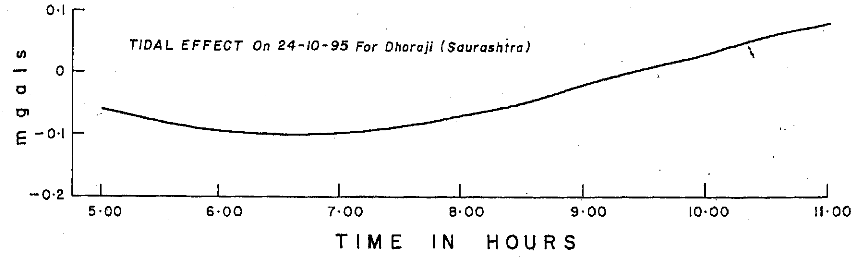

Figure 1. Tidal effect on 24 October 1995 at Dhoraji (Saurashtra).

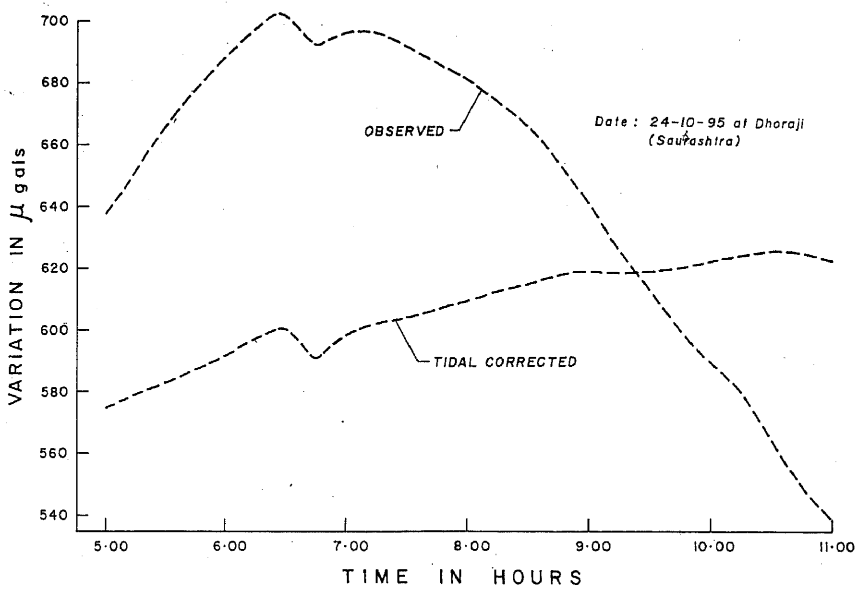

Figure 2. Observed and tidal corrected gravity field at Dhoraji on 24 October 1995.

The recorded variation of the gravity field and the tidal (Figure 1) corrected field on 24 October 1995 for a period from 5.00 am to 11.00 am are shown in Figure 2. Both the graphs show a smooth variation due to short period features like tidal and the drift of the gravimeter over which a shorter period feature of 10–12 μgal between 6.30 and 7.30 am is superimposed. This variation can neither be classified under short period variations due to tidal effect or drift of the gravimeter nor under high frequency noise which have special patterns. Therefore, this variation is highly significant as it occurs with the onset of solar eclipse. It may represent sudden changes in the earth's atmosphere with the onset of eclipse. Exact nature of the changes in atmosphere is difficult to visualize. However, it could be due to decrease in the different kinds of radiations such as gamma rays, X-rays and radio intensity which are found to decrease with the onset of eclipse or simply due to changes in the atmospheric pressure. Therefore to understand its actual nature and mechanism, more planned experiments of this kind should be carried out during solar eclipses throughout the world whenever such opportunities are available.

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Enzyme dot blot assay: A diagnostic tool for detecting rice tungro virus infection

RICE tungro virus (RTV) disease is caused by infection of two different viruses, rice tungro spherical virus (RTSV) and rice tungro bacilli form virus (RTBV) (ref. 1). Both RTSV and RTBV are transmitted in a semi-persistent manner by rice green leaf hopper *Nephotettix virescens*. RTSV infection causes mild or indistinct symptoms. The spherical viral particles are isometric, have a diameter of 30 nm and contain a single-stranded DNA genome of more than 10 kb. RTBV infection, however, causes severity of symptomatology as evident from yellowing of leaves and stunted growth and the symptoms are accentuated in plants co-infected with RTSV[2-4]. The bacilli form particles have a model length of 130 nm and width of 30 nm and can only be acquired by plants from leafhoppers in association with RTSV. The RTBV genome is 8 kb (ref. 5) and is interrupted by two discontinuities which map at specific sites on each strand. Neither RTBV nor its DNA is transmitted mechanically into rice plants. The virus contains two major proteins with molecular weight of 37 and 33 kD.

The method of early detection being mainly serological depends on highly purified viral particles to develop the antiserum. Serological methods, though specific and sensitive enough to detect viruses even in small quantity of samples, the virus purification and obtaining good quality antiserum is rather difficult and resource intensive. Therefore, a study was undertaken to develop simpler, cost-effective technique to detect RTV infection at early stages, using enzyme-based dot-blot assay.

Seven to ten-day-old seedlings of seven genotypes, viz., Taichung (Native) 1, ARC 11554 (IRRI Acc.# 21473), ARC 12596 (# 22176), DWA 8, PTB 8 (# 6201), Palasithari 601 (# 12069), and Utri Merah (# 16682) were inoculated with RTV through the viruliferous vector. Leaf samples were collected after 20 days when seedlings expressed clear symptoms, especially in T(N)1. The samples were extracted in Tris-HCl (0.5 M, pH 6.8), centrifuged (9000 *g*) for 20 min at 4°C and supernatant was used for enzyme dot-blot assay.

Dot-blot assay was performed by blotting the extract onto Whatman No. 1 filter paper by dot-blotting manifold, and staining for alcohol dehydrogenase (ADH), acid phosphatase (ACP), glutamate dehydrogenase (GDH), esterase (EST) and malate dehydrogenase (MDH) (Figure 1). Of the five enzymes studied, ADH and ACP showed difference in activity between the healthy and the infected samples. In case of ACP, the colour changed from brown to blue. Furthermore, depending upon the severity of infection, there was variation in the intensity of colour. Other enzymes such as EST, MDH and GDH did not give consistent results. To check these, proteins were separated on 7.5% non-denaturing acrylamide gel under anionic condition using constant current (20 mA) and gels were stained for ADH and ACP. In both the cases, all the healthy samples showed

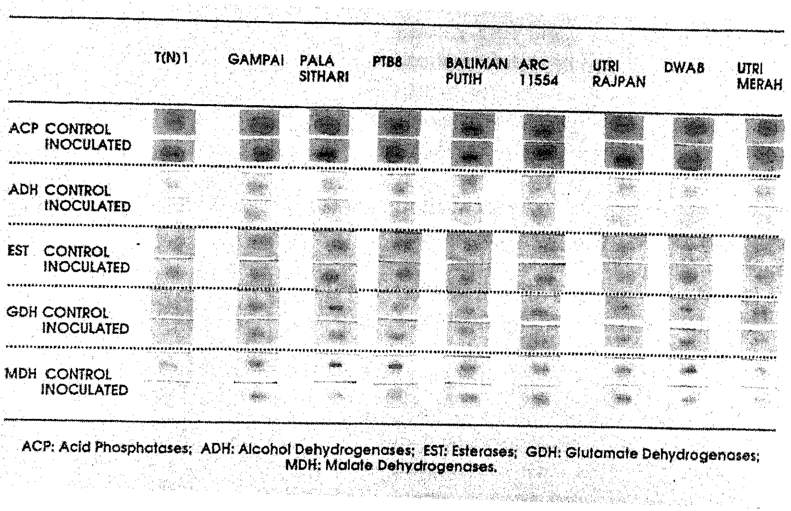

Figure 1. Change in enzyme activities on infection with RTV (20 days after infection).

Table 1. ELISA readings (average of 3 replications) of RTBV and RTSV infected plant samples from seven rice genotypes

Sl. no.	Genotype[a]	RTSV	RTBV	Score[b] Phenotypic	Score[b] Dot-blot
1	Infected (I)	0.64	0.50	+++	+++
	Healthy (H)	0.08	0.06		
2	I	0.56	0.49	+	+
	H	0.07	0.04		
3	I	0.14	0.05	–	+
	H	0.01	0.00		
4	I	0.11	0.35	+	+
	H	0.09	0.03		
5	I	0.24	0.05	++	++
	H	0.01	0.05		
6	I	0.33	0.49	+	+
	H	0.21	0.04		
7	I	0.01	0.34	+	+
	H	0.0	0.06		

[a] 1: Taichung (Native) 1; 2: ARC 11554; 3: ARC 12596; 4: DWA 8; 5: PTB 8; 6: Palasithari 601; 7: Utri Merah.
[b] +: Degree of symptoms expressed; –: No symptoms expressed.

one band and no bands were observed in the infected samples. With other enzymes, the difference was more of nature of change in the intensities of the staining rather than one in the pattern. The difference in colour in the dot-blots was, however, not detectable when sampling was done 7 days after inoculation.

These results were further verified by comparing them with those of ELISA. Lyophilized antisera against RTBV and RTSV obtained from Koganezawa (IRRI) were purified by precipitation with ammonium sulphate and by passing through DEAE cellulose column. Purified antibodies were used for making conjugate with alkaline phosphatase enzyme. Double antibody sandwich ELISA was performed for RTBV (B) and RTSV (S). Average reading of the 'S' and 'B' forms in infected and healthy plants and their phenotypic scores (Table 1) shows that 'B' form showed stronger reaction than 'S' form. Furthermore, infected samples showing higher reading also showed higher degree of change in colour on dot-blots, compared to the healthy ones.

Although this technique being of qualitative nature cannot be compared with techniques such as ELISA, it can be employed as a reliable, low-cost technique for rapid screening of large number of samples.

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Possible occurrence of a Fabry–Perot type of interference filter in a fish eye-lens

The eyes of fish are constructed on general vertebrate plan with cornea, lens and retina. But unlike terrestrial vertebrates, the lens in fish acts as the principal refractive structure, since little refraction occurs at the corneal surface, whose refractive index is approximately equal to that of water.

The function of lens in both 'camera type' and 'compound type' eye appears to be extensive and varied. It may act as a broad band anti-reflection device, may help in reflection of specific wavelength from regions behind the receptive cells, can play a role in selective light absorption and also may act as an interference filter in the eye[1–5]. The optical phenomena exhibited by lens have generally been correlated with the specialization in morphological and anatomical features, and also in organic macromolecular complexes[1–5]. Surprisingly, the possible involvement of inorganic components in these has so far not been explored in detail, although many inorganic elements are known to possess important optical properties with varied applications[6]. The conventional techniques, such as atomic absorption spectroscopy used in the past for elemental analysis of lens and other ocular tissues could not provide information on distribution patterns of elements and their localization in different components of a particular structure.

Electron microscopy–energy dispersive X-ray spectroscopy (EM–EDS), which uses electron optics and characteristic X-rays emitted from elements, appears to be the best device which meets the above criteria. Recent studies from this laboratory could explain some unexplored optical phenomena in relation to the localized distribution of certain elements in fish lens[7,8].

Here we report with the help of scanning electron microscopy and energy dispersive X-ray micro-analysis, a unique structural arrangement of lens components and the localized distribution of the metal, aluminium in the lens, in a way that resembles a Fabry–Perot type of interference filter. The transmission curve of the lens also shows similarity with the said interference filter.

Lens taken out from the eye of a fresh water fish, *Hilsa ilsa* was placed in a carbon-coated brass stub and was cut into two halves with a sharp razor blade. Since the lens is structurally stable, no preparatory technique was needed and it was studied by direct mounting. No mould or embedding medium was used for cutting the hard lens. The sectioned lens was secured to the stub with adhesive tape in such a way that the cut surface would face the electron beam of the microscope. Care was taken to avoid contact of the cut surface with any source of elemental contamination. The samples were air-dried, coated with carbon in a JEE-4X vacuum evaporator (Jeol), and studied in the secondary electron emission and X-ray mode of the scanning electron microscope (JSM-35 CF, Jeol). Energy dispersive X-ray micro-analysis was carried out with EDAX system (AN 10000, Linc Analytical) equipped with a Si (Li) high resolution detector. The operating parameters selected were as follows: accelerating voltage = 20 kV, tilt = 45°, elevation = 0.00°, azimuthal angle = 0.00°. The counting time for each analysis was 100 s, the count rate was 2500 cps and the dead time was 25%. The transmission curve of the lens was obtained using the UV-visible double beam spectrophotometer (U-2000, Hitachi). The sections of lens were placed on a glass cover-slip and the transmittance spectra were obtained. The cover-slips without the lens sections were used as control.

The lens, which is formed by the embryological evagination of an ectodermal cell layer is covered by a thick basement membrane, which is known as the lens capsule. The lens is characterized by the presence of two distinct regions, the central medulla and outer cortex, and the lens substance shows concentric discontinuities suggestive of growth increments (Figure 1). Energy dispersive X-ray micro-analysis with simultaneous visual display by the scanning electron microscope, revealed a characteristic distribution pattern of certain elements in the cortical region (periphery) and the medulla (central part) of the lens. The elements distributed in the medulla are Si, S, Ca, Fe, Cu and Zn (Figure 2 *a*). The cortex, on the other hand, shows the presence of Al, in addition to Si, S, Ca, Fe, Cu and Zn (Figure 2 *b*). The elements were identified from the EDS spectra by noting the energies of the characteristic X-rays of the peak which is specific for a particular element[9]. The unique elemental distribution pattern of the element, Al, in the lens cortex and its total absence from the medulla presents a situation comparable to that of a typical Fabry–Perot type of interference filter. The metal filter or the Fabry–Perot interference filter consists of a layer of di-electric sandwiched between semi-reflecting metal films usually of silver. The construction of a Fabry–Perot interference filter is shown in Figure 3 *a*. The arrangement of the reflecting metal layer, glass-filter and di-electric layer in the system is comparable to the localized distribution of Al, structural arrangement of lens capsule, cortex and medulla (nucleus) of the fish lens in the present study. The glass used in the Fabry–Perot filter is comparable to the capsule surrounding the lens and the di-electric layer of the filter is comparable to the medulla or nucleus of the lens. The reflecting metal layer of aluminium localized in the cortical region of the lens is arranged in a similar fashion as the semi-reflecting metal layer of Fabry–Perot filter, so that the medulla (nucleus) of the lens is sandwiched between the metal films. That Al in the lens exists as some salts and that it does not form any complex with organic macromolecules is clear from the fact that treatment of lens samples with aldehydes and dehydrating solvents like acetone causes leaching of the element. In this context, it is worth mentioning that elements existing as salts are reported to be leached after treatment with aldehydes, acetone, etc.[9]. Further, it may be noted that salts of aluminium are extensively used as reflectors in different optical devices[6]. The transmission curve of the lens, obtained from UV-visible double beam spectrophotometer (U-2000, Hitachi), shows three bands, two around 200–250 nm and the other around 700–800 nm (Figure 4). It is of interest to note that the transmission curve of Fabry–Perot interference filter also shows the presence of only a few narrow wavelength bands[6].

When parallel light rays are incident

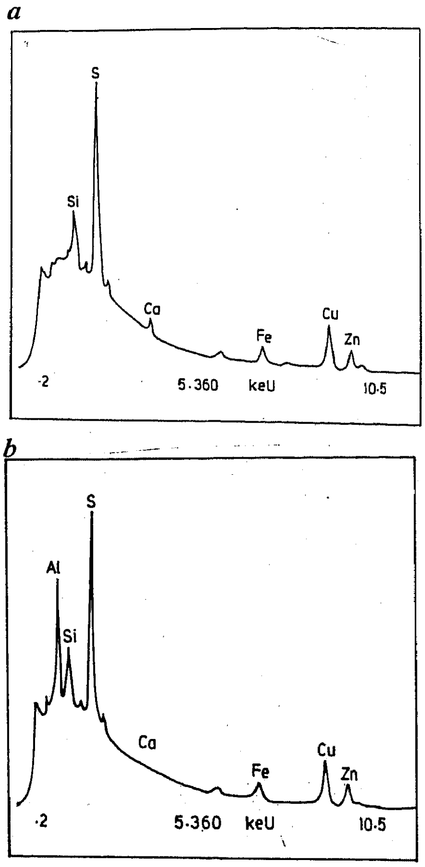

Figure 2. Energy dispersive X-ray spectrum of lens. (*a*) medulla and (*b*) cortex.

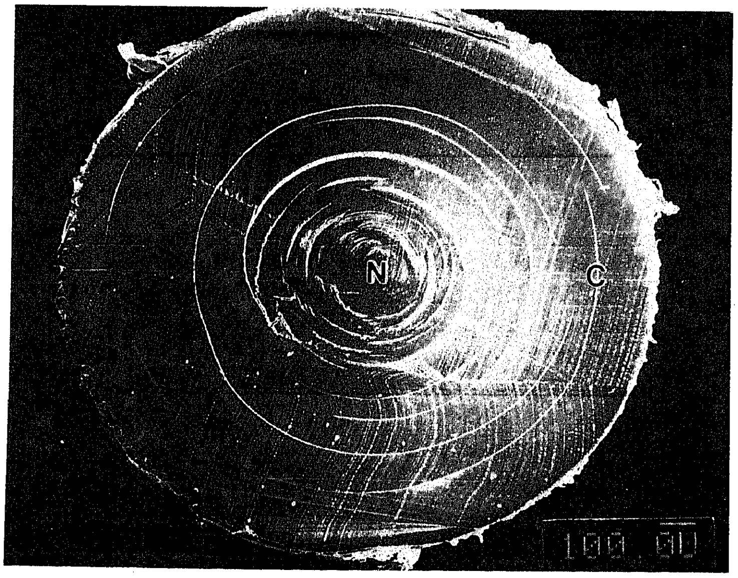

Figure 1. Scanning electron micrograph of TS through lens of *Hilsa ilsa*, showing the medulla and cortex. (N = medulla or nucleus; C = cortex).

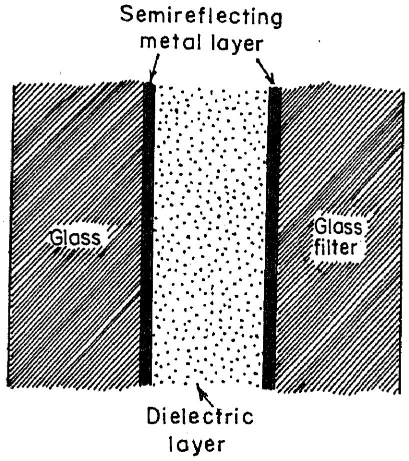

Figure 3 *a*. Diagrammatic representation of the arrangement of a Fabry–Perot interference filter.

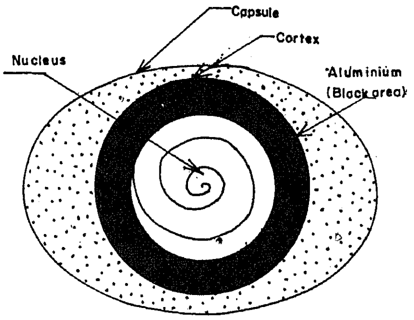

Figure 3*b*. Diagrammatic representation of different components of lens of *Hilsa ilsa* compared to a Fabry–Perot interference filter.

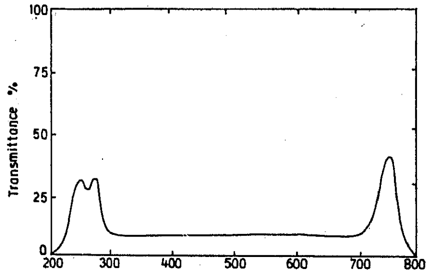

Figure 4. Transmission curve of the lens of *Hilsa ilsa*.

perpendicularly on a system containing Fabry–Perot interference filter, they suffer a substantial reflection as they reach the metal layers. Consequently, most of the rays that traverse the di-electric layer are reflected back. The reflected rays are then superposed on incident rays, which results in a special kind of interference phenomena, termed Fabry–Perot interference. The characteristic feature of this interference is that, it obliterates all wavelengths except a few narrow bands[7].

Although in the absence of experimental data on behaviour, it is not possible to understand the significance of the occurrence of Fabry–Perot type of interference filter in the eye lens of *Hilsa ilsa*, its importance can be explained in terms of the roles played by different types of optical filters detected in fish eye lens[10–11]. Filters are reported to increase visual acuity by reducing chromatic aberration and glare, and also by increasing colour contrast. Although earlier workers believed that fish lenses are achromatic[11], it was later demonstrated that a chromatic aberration of about 5–11 diopters exists in fish lenses, which could be reduced by yellow absorption filters[12,13]. Multilayer corneal interference filters were also shown to function as sun shade against the glare source of downwelling light[14,15].

The Fabry–Perot interference filter detected in the present work, besides acting as a sophisticated sun shade for preventing ocular flare, is expected to play an important role in visual adaptation of the fish, *Hilsa ilsa*. In this context, it is worth mentioning that the fish studied in the present work has a characteristic behaviour of migrating from freshwater to brackish water and vice versa. Such a migration exposes the fish to habitats with very different optical conditions from time to time. The Fabry–Perot type of interference filter which obliterates all but several narrow wavelength bands, may act as an adaptive device for the fish. In the absence of this filter, frequent exposure of the fish to completely different optical conditions could have caused some kind of irregularities in the visual physiology.

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SUDIP DEY

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Transition metal–saccharide chemistry and biology: An emerging field of multidisciplinary interest

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The interactions of transition metal ions with saccharides have been a subject of current interest. This review presents, in brief, the understanding of these interactions through our recent work in this promising field. Methodologies have been developed to synthesize and isolate a large number of complexes of transition metal ions with saccharides, using readily available and/or easy to prepare starting materials. The characterized complexes have been classified into various structurally diverse categories. Biological relevances of some of the transition metal–saccharide complexes have been discussed.

SACCHARIDES are the most abundant class of compounds by weight in the biosphere[1]. Their multihydroxy functionality and well-defined stereochemistry project them as potential candidate ligands for the binding of metal ions[2–7] . The ability to form complexes depends largely on the conformation of the saccharides and the orientation of the OH groups. The interactions between saccharides and metal ions have been known since the turn of the nineteenth century when the first adduct of D-glucose with NaCl was reported[8]. The first comprehensive report of Rendleman Jr.[9], in 1966, deals with the formation of saccharide complexes by the interaction of saccharides with alkali and alkaline–earth metal salts, and metal bases. Notable among the alkali and alkaline earth ion interactions, are the structural analysis of Ca(II)–carbohydrate complexes by Bugg and coworkers[10–14], metal–saccharide adducts reported by Tajmir-Riahi[15–29], and extensive solution studies by Angyal[30–35]. These studies have provided a renaissance of interest in the metal–saccharide interactions. Angyal's studies were primarily directed toward predicting the metal complexing capacities of neutral saccharides, based on their conformations, and the charge and size of the metal ions. The study of transition metal–saccharide interactions has been limited in the literature and largely deals with solution studies of saccharide derivatives of carboxylate, sulphate, amine and other functional groups[36–58].

The interactions between the metal ions and simple saccharides can be considered analogous to the metal–alkoxide interactions. However, it is surprising that the field of transition metal–saccharide chemistry has lagged far behind the corresponding metal–alkoxide chemistry[59,60]. Moreover, as compared to other major polymer classes in biology, viz. proteins and nucleic acids, the saccharide interactions with transition metal ions have received only scant attention[61]. It is highly true that the synthesis and isolation of the complexes in solid state are hampered due to the formation of weak complexes from neutral saccharides because of the high pKa values of the hydroxy groups[62] ($pka_1 > 12$), and, hence, show pH-dependent formation of complexes, particularly under alkaline conditions.

In order to understand the biological roles of essential and toxic metal ions, it is necessary to identify the compounds involved and their possible reactivities, and to elucidate the underlying mechanism through enriching various areas involving bioinorganic, bioorganic and environmental inorganic chemistry aspects[63–66]. The metal-sequestering ability of the saccharides is of interest in the development of novel classes of metal-based affinity chromatography materials, chiral homogeneous catalysts, metal chelators of clinical use, and as models of biologically important chelates[67–71]. The high water solubility of the saccharides, weak immunogenicity and low toxicity makes them useful candidates in developing pharmaceutical agents, e.g. polysaccharide complexes of Mn(II) and Gd(III) as magnetic resonance imaging carriers[72].

Thus the characteristics of transition metal–saccharide interactions were largely unexplored in their solid state complexes and the work has been recently reviewed[6,7,73,74]. Crystallographically-characterized complexes include; a Mo(VI)–D-lyxose complex[75], vanadate–adenosine complex[76], Cu(II) complexes of sugar alcohols[77] and Co(III) and Ni(II) complexes of amino–glycosides[78]. In view of the current interest in transition metal–saccharide chemistry, we have adopted a systematic approach to develop methodologies to synthesize a large number of complexes of the first row transition metal ions with mostly monosaccharides and few disaccharides (Figure 1). The isolated complexes have been characterized in solid and solution states, using several analytical and spectroscopic techniques. The natural occurrence of transition metal–saccharide interactions in biological systems[79–83] has prompted us to study the putative biological relevance of some of these complexes. This review comprises our recent work in transition metal–saccharide chemistry and biology, and is aimed to bring about a better understanding of the transition metal–saccharide interactions.

Coordinating abilities of saccharides towards metal ions

The coordination of polyhydroxy saccharides to the metal ions directly depends upon the spatial arrangement of the hydroxy groups. The metal complexing capacities of saccharides can be predicted simply by knowing some of their characteristics, such as, D or L, α or β, gluco or manno[84]. Thus the most favourable arrangements for polyols are the axial, equatorial, axial (a, e, a), and 1,3,5-triaxial conformations (Figure 2 *a*, *b*). In general, for the six-membered pyranose rings, both *cis* and *trans* diol arrangements are favourable, whereas in the five-membered furanose forms only *cis* diol provides a favourable orientation for complex formation. The saccharides, with open chain systems, possessing a *threo* conformation, exhibit better complexing ability than an *erythro* conformation (Figure 2 *c*, *d*), and the preference for these systems is, *threo* > *erythro* (t > e) for diols and tt > te > ee for triols. However, in saccharides with additional functional groups, like carboxylates, amines and amides, these functional groups are primarily involved in the complex formation.

Synthetic methodology

The rather weak acidic nature of the hydroxyl protons has been a limiting factor for their coordination. Earlier synthetic studies of saccharide interactions with the alkali and alkaline earth metal ions have revealed these interactions to be purely electrostatic, leading to the formation of metal–saccharide adducts[15–29]. We have synthesized transition metal–saccharide complexes, primarily using the sodium salts of saccharides. This method enhanced their reactivity towards metal ion coordination and resulted in the formation of complexes involving hydroxyl groups. Metal halides of divalent, $[NEt_4]_2[MCl_2Br_2]$ and $MCl_2 \cdot xH_2O$ (where M = Mn, Co, Ni, Cu and Zn), trivalent ($FeCl_3$), and tetravalent ($VOCl_2$) metal ions have been used as precursors for the synthesis by the saccharide–sodium salt method in nonaqueous solvents. The complexes of Cr(III) and VO(IV) were synthesized also from the reduction of chromate[85–90] and vanadate[91] respectively, using the saccharides as both reducing and complexing agents in aqueous and nonaqueous media. All the isolated complexes were purified using mixtures of aqueous and nonaqueous solvents to result in low molecular weight and water-soluble complexes. It was found that in some cases, e.g. Cu(II)–saccharide complexes[92], there was an initial formation of the polymeric complexes early in the reaction which later disintegrates to give lower molecular weight complexes. All the attempts to crystallize these complexes were unsuccessful.

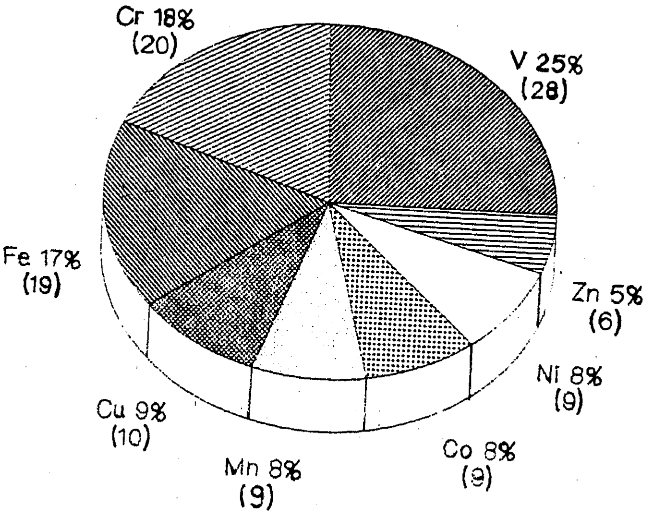
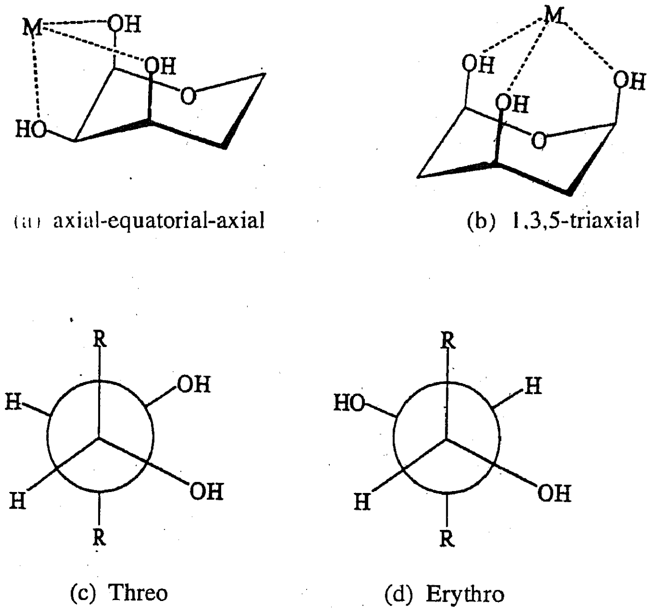


Figure 1. Pie chart showing the number of transition metal–saccharide complexes of each metal type synthesized (as given in brackets), along with percentages.

Spectral, magnetic and electrochemical behaviour

The isolated complexes were characterized by several



(a) axial-equatorial-axial

(b) 1,3,5-triaxial

(c) Threo

(d) Erythro

Figure 2. Favourable arrangements of hydroxy groups of cyclic and open polyols.

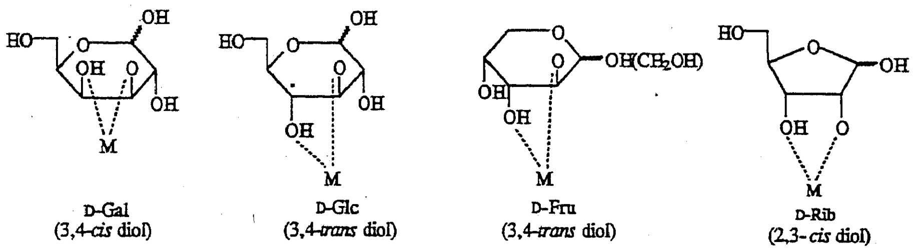


Figure 3. Favourable coordination modes of various saccharides.

spectroscopic and analytical techniques. The similarities found between the diffuse reflectance spectra and aqueous absorption spectra indicated the structural integrity of these complexes in the solid and solution states. Circular dichroism spectra exhibited strong cotton effects in the visible region for VO^{2+} (ref. 93), Co(II) (ref. 94) and Cu(II)–saccharide[92] complexes and a weak cotton effect for Ni(II)–saccharide complexes[95]. An inversion of absolute configuration was observed for D-galactose complexes compared to those of D-glucose and D-fructose. This is attributed to the differences in the orientation of the hydroxy groups on the corresponding saccharides. On the basis of the coordinating abilities of the various saccharides studied, a 3,4-*cis* diol arrangement is proposed for D-galactose complexes and that of 3,4-*trans* diol arrangement for D-glucose and D-fructose complexes. For five-membered rings, *cis*-diol group is the only preferred orientation; D-ribose has been proposed to coordinate via 2,3-*cis* diol groups. The coordination modes of these saccharides are shown in Figure 3. The Cu(II)–saccharide complexes exhibited opposite signs of curves compared to the corresponding VO^{2+}, Co(II) and Ni(II)–saccharide complexes. This is attributed to the ability of Cu(II) to catalyse mutarotation, resulting in the conversion of the saccharide moiety from the stable $^{14}C_1$ chair conformation to the high energy $^1C_4$ conformation[96] as shown in Figure 4.

The Fourier transform infrared (FTIR) studies of the complexes exhibited extensive rearrangement of hydrogen bonding network of the saccharides upon ionization and subsequent complexation. In most cases the presence of α-anomer of saccharides was detected from the FTIR studies[94,95,97]. The information in the far IR region indicated the presence of M–Cl binding in some of the complexes[97]. The ^1H and ^{13}C NMR studies of the complexes in aqueous solution revealed the presence of the open form of oxidized saccharides in Cr(III)–saccharide complexes synthesized by chromate reduction[85–90]. EXAFS studies have shown the primary interaction of the saccharide units through oxygen coordination in the complexes of Cr(III), Fe(III), Ni(II), Cu(II) and Zn(II)–saccharide complexes. The solid state XANES and EXAFS spectra of the Ni(II)–saccharide complexes indicated an octahedral geometry with an ordered interaction between the metal and the saccharide[95]. The Ni...Ni interaction observed in these complexes was found to be shortest in the dimeric Ni(II)–saccharide complexes reported so far[78]. Similar studies with Cu(II)–saccharide complexes supported the presence of divalent copper and indicated the tetrahedral nature of these complexes by binding through two oxo-groups and two chlorides[92]. Satisfactory EPR spectra were obtained in case of Cr(III), VO^{2+}, Mn(II), Fe(III), Co(II) and Cu(II)–saccharide complexes characteristic of their corresponding oxidation states. The studies also indicated a rhombic symmetry in Co(II)- and Cu(II)–saccharide complexes and the presence of spin forbidden $\Delta m_s = \pm 2$ transition, typical for dimers, was observed in Co(II)–D-glucose complex at ~ 10 K with a high spin to low spin cross over[94].

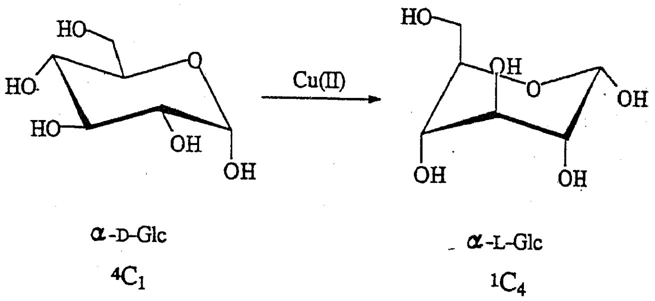


Figure 4. Conversion of $^4C_1$ to $^1C_4$ conformation in the presence of Cu(II) ions.

All the complexes, except for Zn(II)–saccharides, were found to be paramagnetic at room temperature. The variable temperature magnetic susceptibility studies of the complexes showed a weak antiferromagnetic coupling characteristic of hydroxo bridging of the metal centres for Cr(III)[87] and some Fe(III)–saccharide complexes[98,99]. The Mn(II)–D-glucose complex also exhibited a weak antiferromagnetic behaviour in the range 5–300 K for a dimeric nature with a presence of about 5% monomer impurity[100].

The electrochemical behaviour of the complexes was studied by cyclic voltammetric measurements. At the neutral pH, the E_p^c for VO^{2+}–saccharide complexes ranges from – 1.45 to – 1.65 V, whereas for Cr(III) and Fe(III)–saccharide complexes, the E_p^c ranges from – 1.12 to – 1.46 V and – 1.20 to – 1.35 V respectively. These E_p^c ranges may serve as guidelines for addressing metal–saccharide interactions. In the case of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)–saccharide complexes, the metal–saccharide interactions were found to be strengthened under strongly alkaline conditions and almost no complexation was observed under acidic conditions. Intermediate chloro-bound metal–saccharide species were proposed under weakly acidic conditions whereas under highly acidic conditions the complexes completely dissociated to give final anionic metal-chloro species as represented in a scheme for Co(II)–saccharide complexes.

$$\underset{\text{(strongly acidic)}}{[\mathrm{CoCl}_m]^{2-m}} \rightleftarrows \underset{\text{(weakly acidic)}}{[\mathrm{Co(Sacch)}_n\mathrm{Cl}_p]^{2-p}} \rightleftarrows$$

$$\underset{\text{(neutral)}}{[\mathrm{Co(Sacch)}_q]^{x-}} \rightleftarrows \underset{\substack{\text{(alkaline)}\\ (y > x)}}{[\mathrm{Co(Sacch)}_q]^{y-}}$$

Structural diversity

Our efforts in the transition metal–saccharide chemistry have resulted in the synthesis of more than one hundred complexes of first row transition metal ions with mostly monosaccharides and a few disaccharides and the complexes are found to be structurally diverse in nature. The diversity ranges from mononuclear to tetranuclear complexes with few exceptions of polynuclear ones. A metal-wise distribution of all these complexes into various nuclearity categories is shown in a three-dimensional bar diagram in Figure 5. In the synthesis of metal–saccharide complexes from simple, unprotected saccharides, the nuclearity of the complexes turned out to be in 1 : 2 : 1 ratio for mono, di and higher nuclear ones with almost half of the complexes being dinuclear. Formation of dinuclear complexes in the majority is explained on the basis of the available free hydroxyl groups on the saccharides. While all the 28 VO^{2+}–saccharide complexes are exclusively mononuclear, constituting 31% of the total complexes synthesized, the Cr(III) and Zn(II)–saccharide complexes, and a majority of the remaining complexes were predominantly dinuclear, constituting 47% of the total complexes. About one-fifth of the complexes were found to be of higher nuclearity, with tri (14%), tetra (4%) and higher nuclear ones (4%). The geometry of all the first row transition metal–saccharide complexes in the solid state was found to be either octahedral (Cr, Fe, Co, Ni, Cu), or tetrahedral (Mn, Co, Cu, Zn), with the exception of square pyramidal VO^{2+}–saccharide complexes. However, the aqueous solution spectra of all the complexes, including those of VO^{2+}–saccharide complexes, synthesized from VOCl_2 have shown characteristics of six coordination.

The polyhydroxy nature of the saccharides leads to the formation of multinuclear complexes. In the absence of any single crystal X-ray studies of these complexes, a clear picture of the structure of these complexes is only speculative. The metal centres are expected to be bridged by hydroxyls in case of Cr(III) and Fe(III)–saccharide complexes, whereas those synthesized from the metal–halide precursors are expected to be mostly bridged by halides and/or hydroxyl groups. Proposed structures of some of the complexes are shown in Figure 6.

Solution stability

The synthesis of first-row transition metal–saccharide complexes from different precursors and methods has led to the formation of highly water soluble complexes. However, the most striking feature among these complexes is the remarkable hydrolytic stability of VO^{2+}, Cr(III) and Fe(III)–saccharide complexes compared to Mn(II), Co(II), Ni(II), Cu(II) and Zn(II)–saccharide complexes. The saccharide complexes of VO^{2+} synthesized from $\mathrm{VO(acac)}_2$ (refs 101, 102), and those of Cr(III)

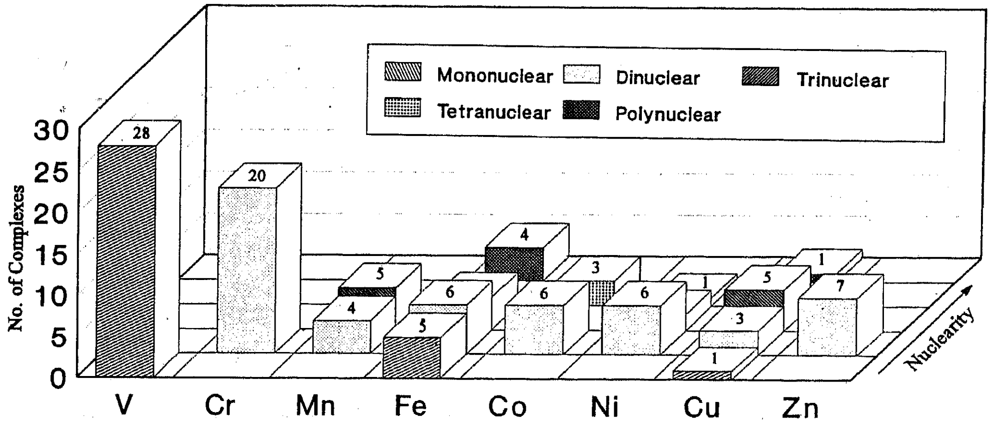


Figure 5. Metal-wise distribution of transition metal–saccharide complexes into different nuclearities.

(ref. 85–90) and Fe(III) (ref. 103) were found to be stable in the pH range 2–12 as studied by absorption spectroscopy and cyclic voltammetry. These complexes exhibited a linear behaviour between the cathodic peak potentials (E_p^c) and pH exhibiting different slopes for different compounds, indicating their relative hydrolytic robust natures. The variable stability of transition metal–saccharide complexes is not only dependent upon the metal ions but also on the nature of the saccharides as illustrated in Figure 7. This behaviour is similar to Irving–Williams series for the stability of divalent first-row transition metal ions[104]. The differences in the stability of saccharide complexes of various metal ions have been explained on the basis of the ionic size of these metals[30]. Generally, the most suited radius for complex formation is ~ 1.0 Å. Therefore, Mn(II) (0.80 Å) forms relatively more stable complexes than Zn(II) (0.74 Å), Co(II) (0.74 Å), Ni(II) (0.72 Å) and Cu(II) (0.69 Å). The higher stability of Fe(III) and Cr(III)–saccharide complexes, in spite of their smaller radii [Fe(III): 0,64 Å and Cr(III): 0.69 Å] is attributed to their higher charge and the involvement of carboxyl groups in the coordination at least in case of Cr(III) complexes. This also follows the complexing ability trend of metal ions in the order, $M^{3+} > M^{2+} > M^{+}$ for a given ligand. The general tendency of the oxo-cations such as those of V and Mo to complex strongly to saccharides[105], explains the unusual stability of VO^{2+}–saccharide complexes.

Relative reducing abilities of saccharides towards metal ions

As mentioned earlier, one of the methods of synthesizing transition metal–saccharide complexes is to reduce the metal precursors using saccharides followed by the complexation. The reducing abilities of the saccharides play a significant role on the availability and transport of toxic and non-toxic metal ions as part of soil biochemistry. These reactions occur at different rates and give rise to a variety of products. We have studied the relative reducing abilities of the various hydroxy containing compounds, including monosaccharides, towards V(V), Cr(VI) and Mo(VI) reductions in acidic medium[106–108]. Based on the quasi-kinetic approach, the relative reducing abilities of various compounds towards these three metal ions were found to follow an order, L-ascorbic acid > L-cysteine > monosaccharides > glycols > ethanolamines. Among the metal ions, the corresponding relative oxidizing abilities followed a trend, Cr(VI) ≫ V(V) > Mo(VI), as expected, based on their

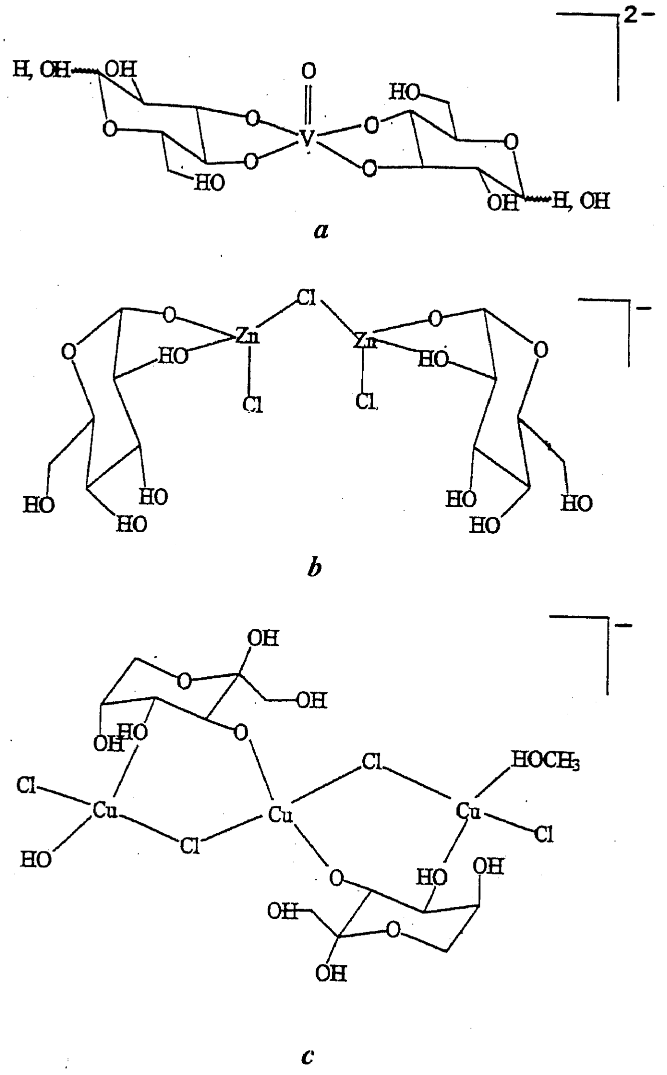


Figure 6. Proposed structures of some selected transition metal–saccharide complexes: *a*, VO–D-Glc complex; *b*, Zn(II)–D-Glc complex; *c*, Cu(II)–D-Fru complex.

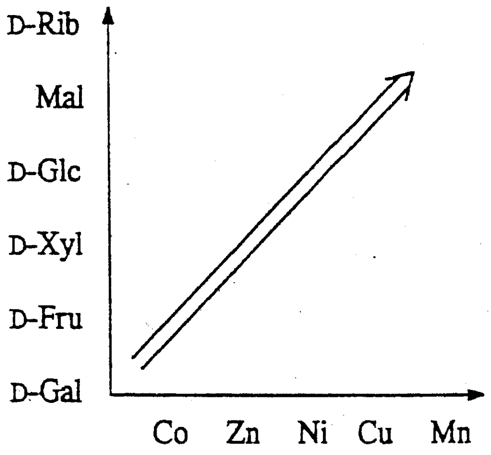


Figure 7. Plot showing the general trend in the solution stability of transition metal–saccharide complexes as dependent on the corresponding saccharides and the metal ions.

reduction potentials. A plot showing the oxidizing ability of these metal ions and the reducing abilities of various reductants is shown in Figure 8, which could be used in predicting the relative reducing or oxidizing abilities. A working model for chromium toxicity has also been proposed[109].

Putative biological relevance

Metal–saccharide interactions are of biochemical importance in relation to absorption, transport and detoxification of metal ions in biological systems. Some enzymatic reactions of saccharides, alkali, alkaline earth and some of the transition metal ions have been suggested to act in cooperation with saccharides. *Vanadobin*, a low-molecular weight compound containing a reducing sugar as the vanadium-binding substance, has been isolated from various tunicates[83]. Low molecular weight saccharide complexes of Ni(II) have been found in mammalian kidneys[81,82]. Thus there is a concrete evidence for the existence and various implications of metal–saccharide complexes in biological systems. Some of the transition metal–saccharide complexes prepared in our laboratory, such as those of VO^{2+}, Cr(III), Fe(III), Cu(II) and Zn(II), have been tested for their putative biological relevances.

The *in vitro* DNA interaction studies with VO^{2+} (refs 91, 102), and Cr(III) (refs 87, 110) and Fe(III)–saccharide complexes have shown them to be potent DNA cleaving agents in the presence of hydrogen peroxide, leading to the formation of open circular and linear forms from the native supercoiled form of DNA (pUC 18 or pSV2 neo plasmid) as demonstrated using gel electrophoresis. A histogram indicating the amounts of different forms of DNA formed due to incubation with vanadium saccharide complexes is shown in Figure 9. Studies carried out in the presence of sodium azide and mannitol have delineated the involvement of oxygen-based species in the DNA cleavage studies. However the ascorbate complexes of these metal ions have shown their DNA cleavage even in the absence of hydrogen peroxide and in their presence these have shown complete conversion of supercoiled form and exhibited more amounts of linear form.

Studies with VO^{2+}–saccharide complexes on the *in vitro* interactions with RNase and DNase exhibited inhibition of RNase but not DNase, suggesting that these complexes could act as transition state analogues (Figure 10) to this enzyme[102]. These complexes also caused lipid peroxidation, cytotoxicity in isolated rat hepatocytes, and were efficient inhibitors of protein biosynthesis in rabbit reticulocyte lysates. As the soluble vanadyl–saccharide complexes are found to be toxic to cells, even if these are found to be good in reducing the blood sugar levels,

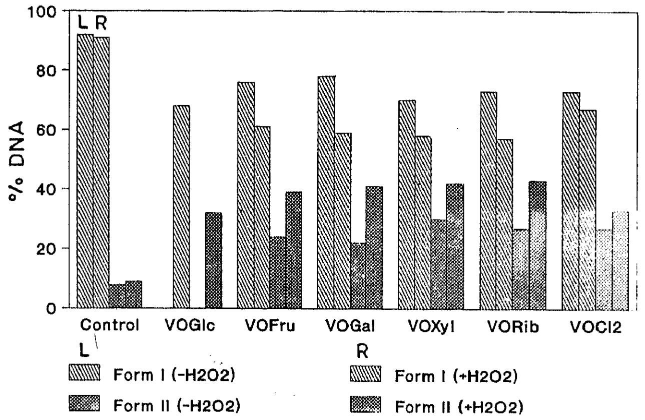


Figure 9. Effect of VO^{2+}–saccharide complexes of D-Glc, D-Fru, D-Gal, D-Xyl, D-Rib on pUC-18 DNA, compared to $VOCl_2$, showing percentages of Form I (supercoiled) and Form II (nicked) in the presence and absence of H_2O_2.

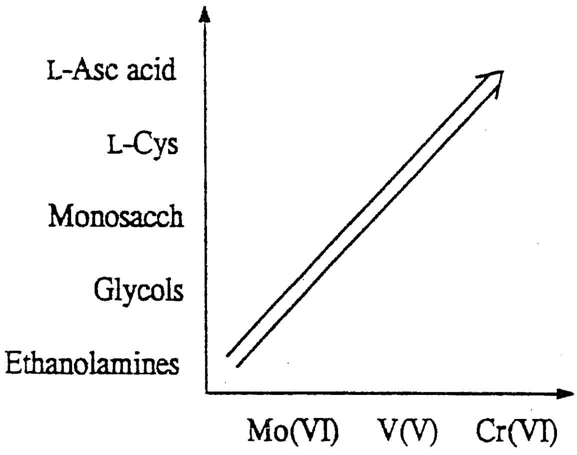


Figure 8. Plot showing the general trend in the oxidizing abilities of Mo(VI), V(V) and Cr(VI) towards various reducing agents.

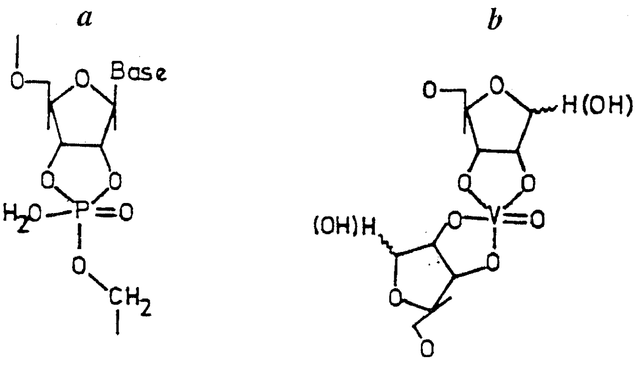


Figure 10. *a*, Transition state complex in the RNase-catalysed hydrolysis of RNA, and *b*, proposed schematic representation of complex $[VO(D\text{-}Rib)_2]^{4-}$.

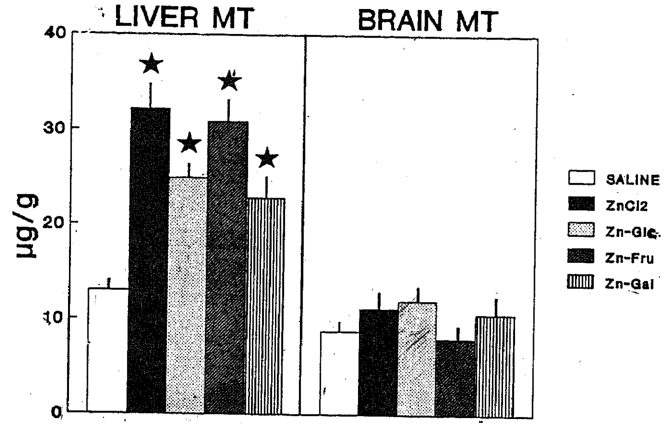


Figure 11. Effect of Zn(II)–saccharide complexes on rat liver and brain metallothionein (MT) levels compared to $ZnCl_2$ at a dose of 5 Mg/kg body weight in all the cases.

their use as oral insulin mimic is debatable. However, these complexes can perhaps be developed as specific agents of DNA cleavage.

The Fe(III)–D-glucose complex showed ATP-dependent intestinal absorption in rats, and was found to be relatively better absorbed than the free salt as studied using the everted sacs of rat intestines. The iron complexes have also been demonstrated for their controlled reductive release of iron in the presence of sodium dithionite as reducing agent using UV-Vis absorption and cyclic voltammetry experiments.

The Cu(II) (ref. 92) and Zn(II)–saccharide[97] complexes were found to significantly induce *in vivo* metallothionein synthesis in mice liver but not in brain. Corresponding data is shown in Figure 11 through histogram in case of Zn–saccharide complexes. The effect of these complexes was comparable to that of their corresponding salts even at very high concentration doses without showing any sign of toxicity. Further studies on the effect of Zn(II)–saccharide complexes on the δ-aminolevulinic acid dehydratase (ALAD) activity, urinary δ-aminolevulinic acid (u-ALA), blood zinc protoporphyrin (ZPP) and glutathione (GSH) levels suggested these to be potential oral Zn supplements[111]. These complexes were also capable of restoring the lead-induced biochemical disorders (ALAD activity, u-ALA, GSH levels) in lead poisoned male rats. All these biological studies reveal that the transition metal–saccharide complexes have some biological significance and promise to be of potential medicinal/clinical value.

Implications

The transition metal–saccharide interactions have wide occurrence in nature and regulate the bioavailability of metal ions through various primary and secondary interactions. Though a large volume of solution studies in the literature have demonstrated the complex formation, our group has been instrumental in developing the subject of transition metal–saccharide chemistry and biology. In the process, a plethora of low-molecular weight, water soluble, first-row transition metal–saccharide complexes are synthesized and characterized, but the real lack of crystal clear demonstration of metal–saccharide interactions through X-ray structures undermines the contributions made in this field. Some of the main reasons for non-crystallization of these complexes arise not only from the anomeric nature of the saccharides, but also due to the involvement of free-hydroxyl groups in various interactions, including those of ion (counter cation) . . . dipole type, leading to the formation of extensive aggregations. Such futile interactions can be prevented by selecting saccharides in their specific derivatized forms and to further enhance the crystallizable characteristics of the transition metal–saccharide complexes. The efforts in this direction are currently underway in our laboratory. The X-ray crystal structures and catalytic applications of some transition metal complexes with derivatized saccharides are reported in the literature[112–118]. The role of saccharides in the reduction and complexation of metal ions has been understood and the biological relevance of transition metal–saccharide complexes is of growing interest in the future. Given the importance of transition metal–saccharide interactions in agricultural, industrial, and pharmacological applications[6], the field of transition metal–saccharide chemistry and biology attracts attention of scientists with diverse interests.

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Derivatization of heteroditopic cryptands: A new generation of amphiphiles

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Multi-gram scale synthesis of a few heteroditopic cryptands have been achieved. These cryptands have three derivatizable secondary amino groups which could be derivatized with acid chlorides to form a new generation of amphiphiles. These amphiphiles readily form vesicular structures and monolayers as well.

BIOMEMBRANES[1] provide one of the most important structural units in the organization of the biological cells. Synthetic molecules containing a hydrophilic headgroup and one or more tails (amphiphiles) are known to form a great variety of supramolecular structures such as micelles, monolayer, bilayer, multilayer rods and vesicles. Surfactant monolayers, bilayers and phospholipid vesicles have been used extensively as membrane models. In addition, such systems are useful in drug delivery and targeting[2], medical imaging[3], catalysis[4], energy conversion[5] and separation[6]. Furthermore, highly charged synthetic bilayers offer a challenge to colloidal chemists. They are useful to test models for colloidal stability, electrical double layer and amphiphilic self-assembly.

In 1976, Kunitake *et al.* reported[7] for the first time a totally synthetic vesicular structure. Since then a large number of synthetic vesicles have been reported with different chemical structures and composition of hydrophilic headgroup(s) and hydrophobic tail(s). The examples include single tail surfactants with rigid segments[8], a surfactant with a hyperextended chain[9], a two-headed single-chain surfactant[10], two-headed two-tail surfactants[11], surfactants with identical hydrophobic chains and two hydrophilic headgroups connected through a spacer[12] and double-chain double-headgroup surfactants containing one cationic and one anionic headgroups[13].

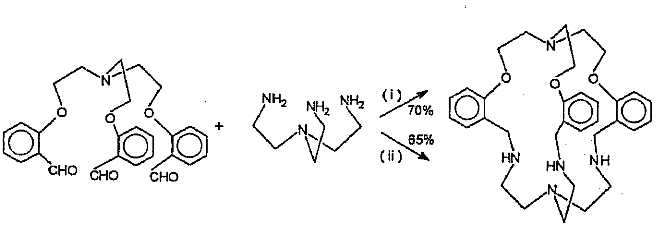
Formation of monolayer and multilayer films by the Langmuir–Blodgett technique has assumed greater importance in recent times with the current demand for materials with tailored interfacial properties. Langmuir monolayers incorporating different types of monocyclic compounds[14–17] have been reported in the literature. Fullerenes[18,19] have been studied in Langmuir troughs as such, or mixed with other molecules for the possible formation of monolayers at the air–water interface. The macrobicyclic benzo[2.2.2] Na-cryptate with covalently linked fullerene (C_{60}) as the hydrophobic moiety is also available in the literature[20].

Cationic surfactants have a spectrum of biological activity, including antibacterial and immunosuppressant properties[21]. Synthesis of analogous molecules with higher charges on the head group is important as these synthetic amphiphiles can be used as anti-parasitic agents and finally as anthelmintic agents[22] owing to their detergent actions.

We became interested in synthesizing cryptand, with long alkyl chains attached as side-groups, for having new generation amphiphiles which would lead to yet another class of vesicular structures and mono- and multi-layer films. Cryptands[23] have proved their usefulness in many areas of chemistry and biochemistry such as molecular recognition, catalysis, transport and molecular devices. In a cryptand, the donor atom's topology can be varied through ligand design. The information thus stored in the form of three-dimensional disposition of donor atoms can be read out by a specific metal ion or a molecular guest. This phenomenon of molecular recognition has become a prominent field of research. It not only serves in the understanding of biological functions but also offers a key in the development of new types of functionalized materials. Cryptand-based amphiphiles can form organized molecular assemblies, incorporating metal ion/molecular recognition sites. Such systems are expected to have characteristics quite different from homogeneous media. They are expected to be useful in understanding biological membranes, in the development of novel two-dimensional molecular assemblies, in fabricating new generation molecular devices[24] etc. Furthermore, when a metal ion is included inside the cavity, the resulting charged cationic surfactant can have a spectrum of biological activity which includes antibacterial and immunosuppressing properties.

Synthesis of cryptands and their derivatization

Since the first synthesis of cryptands by Lehn[25], various procedures have been adopted to synthesize these molecules. Stepwise synthesis is one of the most commonly used methods and involves the generation of an intermediate macrocycle followed by bridging with another chain to yield the macrobicycle. However, more often than not the yields of the desired products are quite poor. The principal problem encountered during the synthesis has been the oligomerization of the reactants leading to lower yields. Besides, the desired products are often mixed with impurities from which the pure product cannot be isolated easily. We have adopted a new strategy[26,27] in synthesizing these molecules. The method involves coupling of two tripodal units by [1 + 1] Schiff base condensation which is the more direct route and involves the formation of three bonds in one pot synthesis. The logistics behind our synthetic approach has been to use a large alkali metal ion like Rb^+ or Cs^+ as a template (Scheme 1) where the two tripodal units can converge. The usual high-dilution condition is thus not necessary. Moreover, to expedite the condensation reaction, expecting that the side products will be minimized, the temperature of the medium was elevated to ~ 40°C. These reaction conditions lead to high yields of the desired cryptand in pure form. Subsequently, another strategy[28–30] has been adopted for the synthesis of these molecules that affords comparable yields. This strategy involves low temperature condensation (Scheme 1) of the two tripodal units under moderate dilution without using any templating metal ion. Following the lead by Smith and coworkers[31], we have found that if the two tripodal units are allowed to react at 5°C, the reaction proceeds smoothly without a templating metal ion. However, the solvent system for the condensation reactions is quite crucial in that only a particular solvent system gives better yields. In our case, MeOH : THF (40 : 1 v/v) is found empirically to be the solvent of choice. This method works well with a number of different tripodal units. We believe that at low temperature, the tripodal units have significantly less movement of the tripods and can converge to give the product in high yields. The rate of Schiff base condensation reaction is not adversely affected due to lowering of the temperature. Figure 1 shows three X-ray crystallographically



(I) Template synthesis: 40°C, MeOH, CsCl, $NaBH_4$

(II) Low temperature synthesis: 5°C, MeOH+THF, $NaBH_4$

Scheme 1. Synthetic strategy for the heteroditopic cryptands.

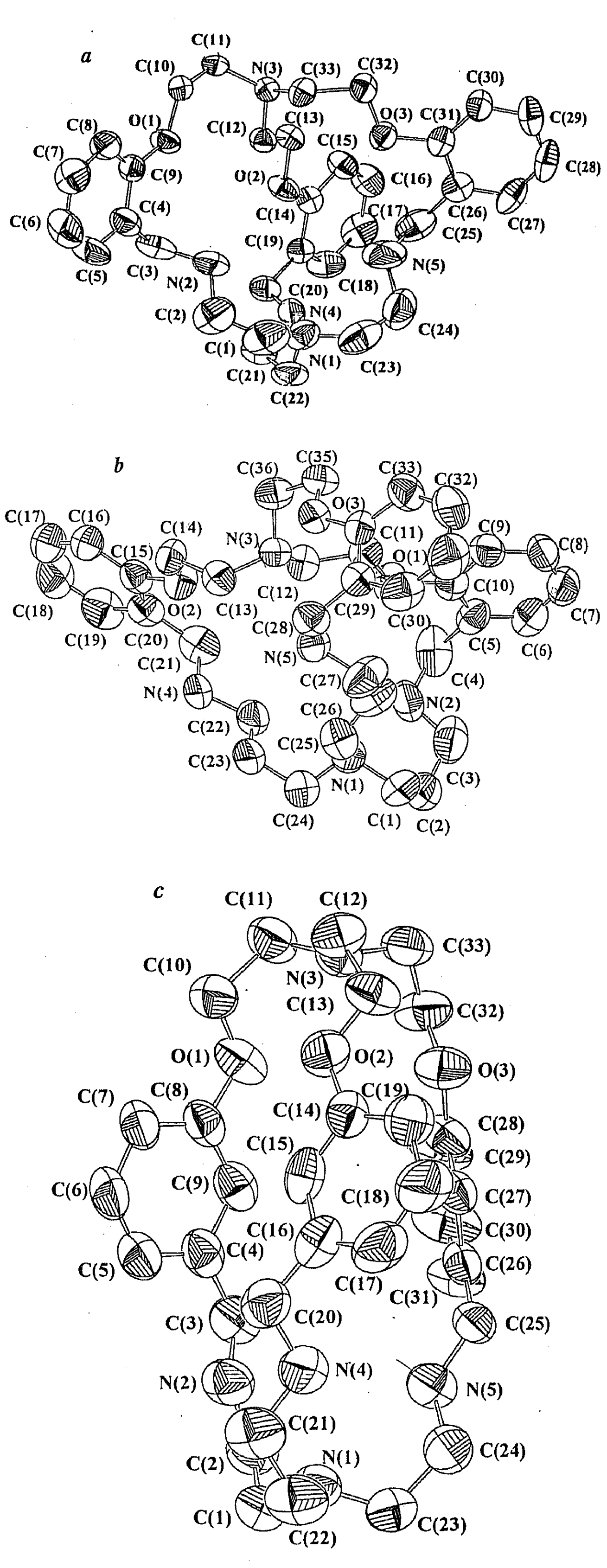
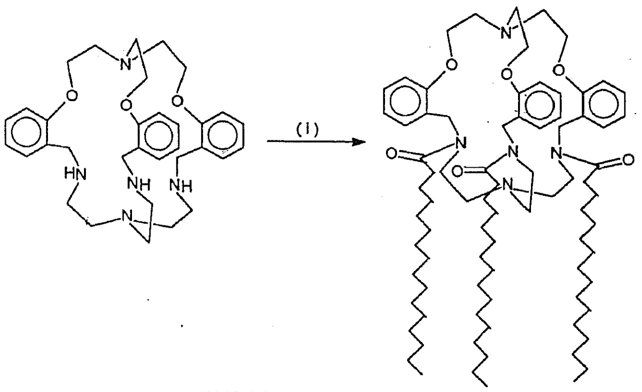


Figure 1 *a–c*. An ORTEP drawing of heteroditopic cryptands showing the thermal ellipsoids and the atomic numbering scheme. Hydrogen atoms are omitted for clarity.

characterized[28-30] cryptands, synthesized at low temperature in multi-gram scale quantities. This method has also been used successfully for the [2 + 3] condensation reactions[32] as well where two tripods and three spacer units are involved. When the two tripodal units have different sizes, they undergo [2 + 2] Schiff base condensation to generate macrotricycles[33]. However, it is now realized that not only the size mismatch but the conformational rigidity of the tripodal units is also important to drive the reaction to a particular product[34]. It is of relevance to mention here that low-temperature synthetic strategy is superior to that of the high-temperature one for multi-gram scale synthesis of the cryptands.

Each of the cryptands synthesized has at least three secondary amino groups in their framework which can be functionalized. To have amphiphiles[35], each cryptand is treated with an acid chloride (Scheme 2) in 1 : 3 molar ratio in dry THF medium under an Argon blanket. The reaction goes cleanly and almost quantitatively. The ^{1}H-NMR spectra of the amphiphiles are consistent with their structures and the FAB-mass spectra exhibit the molecular ion peak (100%) in all cases. These cryptand-based triple-tailed triple-head amphiphiles represent a new generation of surfactants.



(i) $Me(CH_2)_{14}COCl$, NEt_3, THF

Scheme 2. Synthetic strategy for the amphiphile.

Formation of vesicles by cryptand-based metal-free or complexed amphiphiles

We have reported that a cryptand based triple-head triple-tailed amphiphile, where the alkyl chain has fifteen carbon atoms, readily forms stable vesicles[35]. When copper(II) perchlorate hexahydrate salt is added to a methanolic solution of the amphiphile, the Cu(II) complex is formed. The coordination of Cu(II) within the cryptand cavity can be established by its EPR and electronic spectral signatures. The amphiphile with Cu(II) inside also readily forms vesicular structures[35]. Average diameter of the vesicles (Figure 2) ranges between 350 and 450 nm. Since the first report, a number of new amphiphiles have been found to be able to form vesicular structures. However, the stability of the structures depends upon the alkyl chain length[36]; the stability of

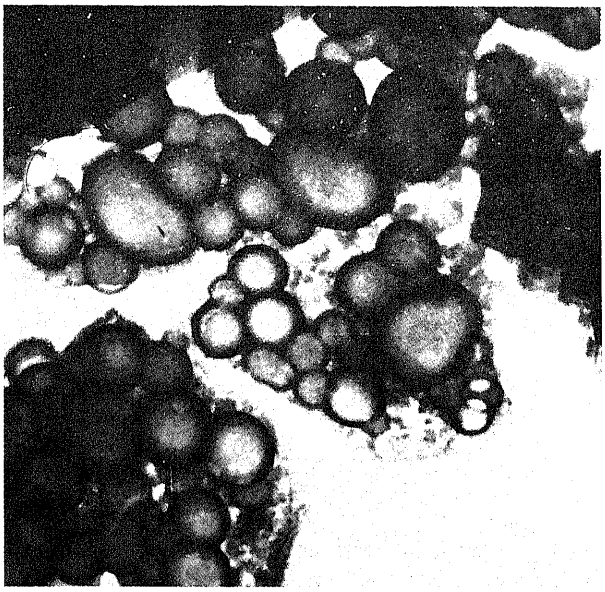


Figure 2. Negative-stain transmission electron micrographs of vesicles of a new generation amphiphiles (× 20000).

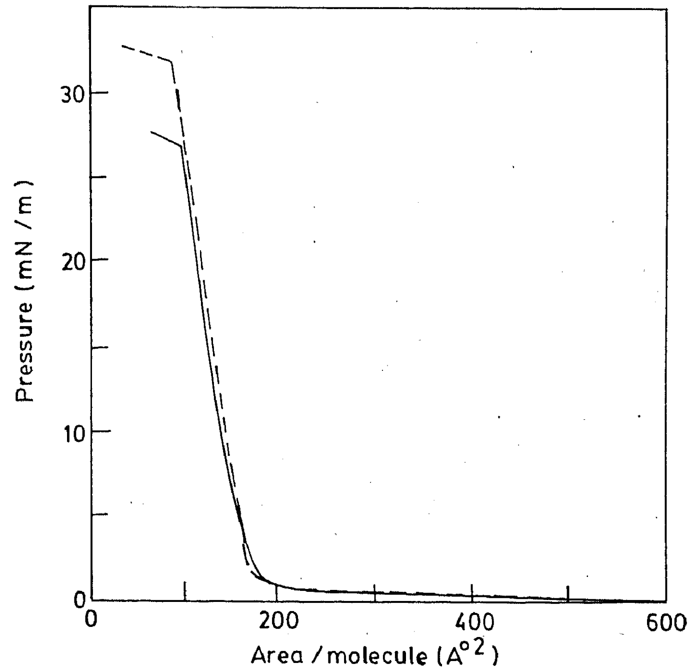


Figure 3. Pressure-area (Π–A) isotherm of a metal-free amphiphile (solid line) and complexed amphiphile (broken line), at 298 K.

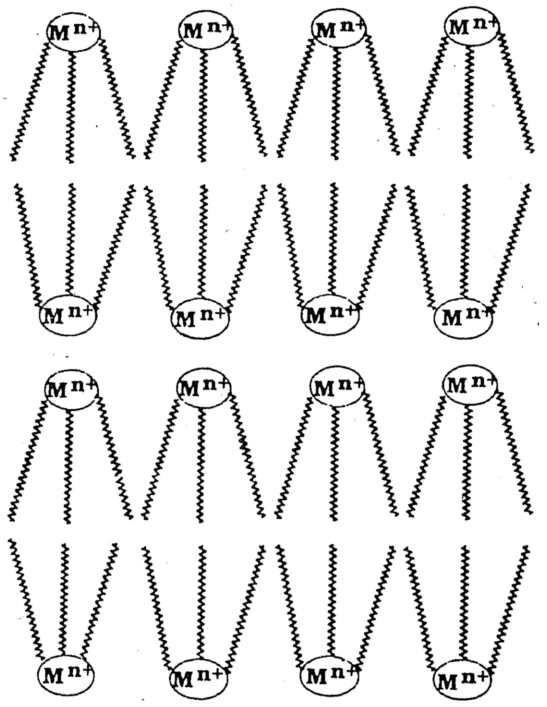


Figure 4. An illustration of a multilayer LB-film with metal ions included in the cavities.

free and complexed amphiphiles decreases with the lowering of carbon chain length. No correlation can be made, however, regarding the vesicular size and alkyl chain lengths from the electron micrographs.

Formation of monolayer by cryptand-based metal-free or complexed amphiphiles

It is of interest to see whether cryptand-based amphiphiles can form stable monolayers at the air–water interface. When probed with the tris-palmitoyl derivative[35] of the cryptand as illustrated in Scheme 2, in a Langmuir trough at 298 K, a well-defined isotherm[37] (Figure 3) is obtained. Subsequently, a number of amphiphiles with varying chain lengths as free or metal complexed are found to exhibit isotherm as in Figure 3. The stability of the films are found to be dependent on the length of the alkyl chain. As the chain length increases, the monolayer becomes more stable whether free- or metal-complexed. However, it was not possible to transfer any of the monolayer films by vertical dipping onto a variety of substrates, e.g. glass, polycrystalline Pt or Au, glassy carbon, etc.

Conclusion and future scope

It is now realized that cryptand-based amphiphiles form stable vesicular structures as free- or as metal-complexed states. It is to be probed whether they can form vesicular structures spontaneously. This would be an important study as suitably designed cryptand-based amphiphiles can be potentially useful in catalysis and in charge separation to harvest solar energy. Preliminary studies in our laboratory show that the metallo-amphiphiles can cause death to certain parasites and ultimate disintegration of their cell-wall. A likely mechanism[22] for this type of effect is the destabilization of the tegumental membrane bilayer due to detergent action of metallo-amphiphiles.

The propensity with which these amphiphiles form Langmuir monolayers can be made use of to have new materials. If it is possible to get stable LB-films with paramagnetic metal ion(s) inside the cavity, it will lead to a structure where we can have two-dimensional paramagnetic layer followed by diamagnetic spacers (Figure 4). Such magnetically anisotropic systems are of enormous importance in materials research. Some of these potential uses of cryptand-based amphiphiles are being probed in our laboratory.

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Binuclear iron, manganese and copper centres in biology: Synthetic analogue approach

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Identification of binuclear (oxo-/hydroxo-/carboxylato bridged) iron, manganese and copper centres of biology are of comparatively recent origin. Noteworthy among them and of relevance to this article are: (i) the marine–invertebrate respiratory protein, hemerythrin (bridged diiron centre), (ii) oxygen-evolving centre of photosystem II (bridged manganese centres), (iii) manganese-containing catalase, pseudocatalase and ribonucleotide reductase (bridged dimanganese centres) and (iv) dicopper centre of tyrosinase. The presence of such bridged centres in biology has inspired bioinorganic chemists interested in synthetic model studies to synthesize and characterize such biological motifs in the laboratory, using designed ligands. Emphasis is directed toward their molecular structure, electronic structure and functional properties to compare with that present in biology. This article summarizes the results obtained in the author's laboratory along these lines.

The field of bioinorganic chemistry[1,2] is burgeoning and the problems of this rapidly expanding field are some of the most fascinating ones at the interface of chemistry, biology, agriculture and medicine. Hence scientists from a variety of disciplines are attracted to this field.

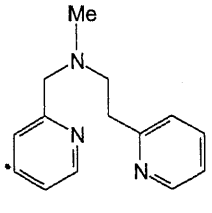
A principal theme of bioinorganic chemistry is the design of metal complexes which mimic active sites in functional metalloproteins. The veracity of these synthetic mimics can be measured by the congruence between their spectroscopic, structural and chemical properties and those features of the metalloproteins. The synthetic analogue approach[3,4] is based on the premise that the chemistry of the metal binding site is dependent, for the most part, on the immediate coordination environment of the metal ion. For most metalloproteins, the immediate coordination environment consists of donors from the side-chains of amino acids. Sometimes, a prosthetic group (e.g. a porphyrin ring) completes the coordination sphere of the metal ion.

Our own involvement in this area began in 1989. Our studies have been aimed at mimicking both the molecular and electronic structural aspects (however, electronic structural in particular) and in some cases functional aspects of the relevant proteins or enzymes, using designed ligands. We have used magnetic, spectroscopic and electrochemical techniques to determine the structures of the binuclear model complexes. These synthetic binuclear complexes have helped/augmented our knowledge in understanding the physico-chemical properties of the natural systems (see below).

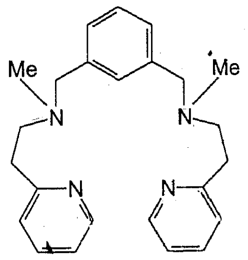
In this article we present a brief summary of the current status of our synthetic models for the non-heme oxygen-transport iron protein hemerythrin (and its ruthenium analogue), water-oxidizing manganese complex of the photosynthetic apparatus and a binuclear copper monooxygenase (tyrosinase) that hydroxylates aromatic phenols.

The ligand design approach

Ligand design plays a key role in mimicking active sites of metalloproteins. Here a chelating ligand is viewed as a collection of adjustable components. These components are: (i) the donor atoms and their basicities; (ii) the donor functional groups and their spatial orientation and electronic and steric properties; (iii) the chelate rings and their sizes, structures, strains, and relationships to each other; (iv) the ring substituents and their electronic and steric effects; etc. In essence, ligand design can be viewed as the process by which the components are varied to control the properties of a targeted system. The synthetic model studies presented here use two pyridine-rich ligands: a tridentate nitrogen-donor ligand, having a five-membered and a six-membered chelate ring (L^1), and a binucleating ligand providing two terminal nitrogen coordination having six-membered chelate rings to each metal centre connected by a *m*-xylyl spacer (L^2).



L^1



L^2

Binuclear iron centres in biology

O_2-carrier protein hemerythrin and structures of its various forms

Hemerythrin is the oxygen-carrier protein found in marine invertebrates, containing two nonheme iron atoms per subunit. It is prototypical of an emerging class of oxo-bridged non-heme iron proteins and enzymes[5–10]. It functions in a manner directly comparable to the mammalian proteins myoglobin and hemoglobin. In the reduced deoxy form, the core is asymmetric (Figure 1), having one five and one six-coordinate iron atom. Two protein carboxylates and one solvent-derived hydroxide bridge link the two iron atoms. In the met form, the bridge is deprotonated. Terminal ligation sites are occupied by nitrogen atoms from the imidazole side chains of five histidine residues, and in the met and oxy forms by an additional exogenous ligand. As dioxygen approaches the diiron centre, it binds to the five-coordinate site. Electron transfer then occurs with oxidation of both ferrous ions to ferric and reduction of the dioxygen to peroxide. This is accompanied by transfer of the hydroxo proton to the peroxide to give a hydroperoxide ion (Figure 1).

Developments in hemerythrin model studies

Since the initiation of the hemerythrin model studies[5–10] by Lippard *et al.* and Wieghardt *et al.* in 1983 there has been a growing interest in the synthesis of triply bridged (μ-oxo)bis(μ-carboxylato)diiron(III) complexes using a variety of tridentate nitrogen, containing facially capping ligands. The synthetic strategy adopted has mainly been the 'self-assembly' method of Ibers and Holm[3]. Some workers also used basic ferric acetate as their starting materials. However, our approach[11] resembles that of Lippard *et al.* using preformed $[Fe_2OCl_6]^{2-}$ ion.

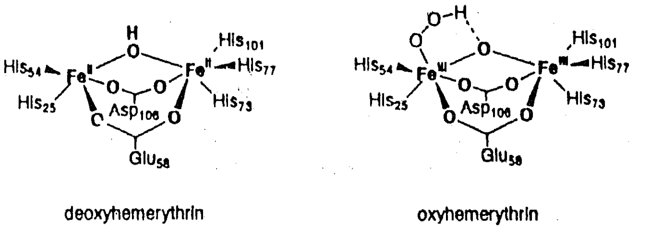
Using an unsymmetrical triamine ligand L^1, we synthesized[11] an orange-brown compound $[Fe_2(\mu\text{-}O)(\mu\text{-}O_2CMe)_2(L^1)_2][ClO_4]_2\cdot 2H_2O$ (**1**). The complex **1** exhibits infrared [bridging acetate groups, asymmetric υ(FeOFe) vibration mode, water of crystallization, and vibrations due to ionic perchlorate], electronic (Figure 2) and Mössbauer spectral features similar to those of structurally characterized μ-oxo-bis(μ-carboxylato)diiron(III) complexes. The temperature-dependence of the magnetic susceptibilities of **1** is as expected for strongly coupled S = 5/2 dimers. The inequivalence in the chelate rings around each iron(III) might have caused the asymmetry in this compound which is reflected in its distinctively strong antiferromagnetic coupling ($J = -125\ cm^{-1}$) (ref. 11).

The lability of the bridging acetate groups in **1** has been demonstrated[11] by exchange with diphenyl phosphate (eq. (1)) as revealed by UV-vis spectroscopy (Figure 2). This study has relevance to the functioning of purple acid phosphatases[7].

$$[Fe_2(\mu\text{-}O)(\mu\text{-}O_2CMe)_2(L^1)_2]^{2+} + 2(PhO)_2PO_2H \rightarrow \{Fe_2(\mu\text{-}O)[\mu\text{-}(PhO)_2PO_2]_2(L^1)_2\}^{2+} + 2MeCO_2H. \quad (1)$$

Interestingly, we synthesized[12] a ruthenium(III) analogue of complex **1**, $[Ru_2^{III}(\mu\text{-}O)_2(\mu\text{-}O_2CMe)_2(L^1)_2][ClO_4]_2\cdot 4H_2O$ (**2**). From the success of hemerythrin core model studies, others[13–16] have also synthesized this kind of ruthenium analogues. In dry acetonitrile solution complex **2** can be oxidized by $1e^-$ using constant-potential electrolysis or chemical oxidation by Ce^{4+}. The one-electron oxidized species $[Ru_2^{III,IV}(\mu\text{-}O)(\mu\text{-}O_2CMe)_2(L^1)_2]^{3+}$ reverts to complex **2** by the addition of water, implying oxidation of water. This reaction is presented schematically in Figure 3.

Thus we have synthesized an accurate electronic structural model for the met form of hemerythrin (for example, metazidohemerythrin). Analogous diruthenium(III) complex when oxidized by one electron is capable of oxidizing water. This property is important for understanding the water oxidation mechanism.



deoxyhemerythrin

oxyhemerythrin

Figure 1. X-ray crystal structures of deoxy- and oxyhemerythrin.

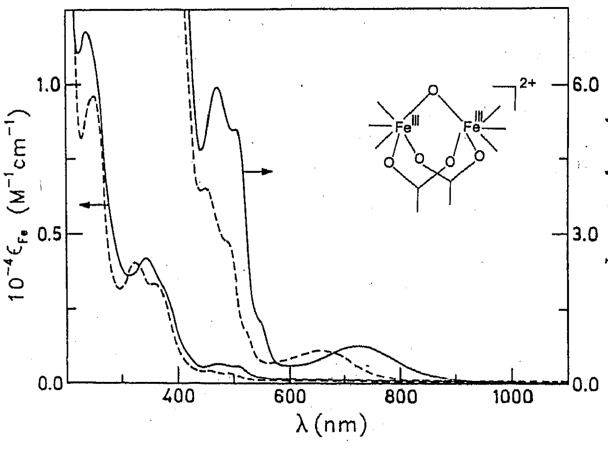


Figure 2. Acetate exchange reaction of $[(L^1)_2Fe_2^{III}(\mu\text{-}O)(\mu\text{-}O_2CMe)_2][ClO_4]_2\cdot 2H_2O$ with diphenyl phosphate as monitored by absorption spectroscopy in acetonitrile solution (——) before and (– – –) after addition of 2 equivalents of $(PhO)_2PO_2H$.

Coupled manganese centres in biology

Photosynthetic apparatus[17–27]

The most important role in nature for the metal manganese is its involvement at the water oxidation/oxygen evolution centre within the photosynthetic apparatus of green plants and cyanobacteria. In the water-oxidizing photosynthesis, generation of chemical energy from light, proceeds by light-induced, transmembrane charge separation of protons and electrons at the reaction centres photosystem I (PS I) and photosystem II (PS II), during which the water is oxidized in the active site of PS II.

$$2H_2O \rightarrow O_2 + 4H^+ + 4e^- .$$

The mechanism of oxygen evolution in PS II is typically discussed in terms of the so-called S-states. The model is based on the measurement of oxygen release after a series of short flashes of light, so that, in each reaction centre, there is a single charge separation event taking place per flash. The yield of O_2 in response to short flashes of light shows a characteristic oscillation pattern with a periodicity of four flashes. To account for this result, it was proposed that PS II cycles through five states $[S_0–(S_4)]$, the index of which refers to the number of oxidizing equivalents stored (Figure 4).

The intermediate states are each defined by the number of electrons extracted from the water-oxidizing complex of PS II. The species which is oxidized in each of the S transitions appears to be a tetramanganese cluster, except possibly for the $S_2 \rightarrow S_3$ transition. Here EPR evidence has implicated a protein residue close to the manganese cluster, possibly a histidine residue. One essential feature of the above scheme is that, during the $S_0 \rightarrow S_3$ transitions, water is not oxidized. Water oxidation apparently takes place only after the third step, in one concerted four-electron reaction or two two-electron reactions.

Structure of the O_2-evolving centre in photosystem II

It is generally agreed that, stoichiometrically, four manganese ions are associated as an indispensable cofactor with each PS II unit, but the question of how the metal ions organize within the protein is still the subject of considerable debate.

Recently, Klein and co-workers[25] have proposed from their EXAFS experiments a structural model which is summarized in Figure 5. The active site would contain two di-μ-oxo units linked by a μ-oxo-bis-μ-carboxylato bridge. The Mn–Mn distances would be $Mn_a–Mn_b = Mn_c–Mn_d = 2.7$ Å and $Mn_b–Mn_c = 3.3$ Å.

Dinuclear manganese centres in biology[8,22–24,27]

Recently, proteins with proposed (μ-*oxo*)dimanganese active sites have emerged. In this group are the ribonucleotide reductases found in coryneform bacteria, and catalases found in aerobic lactic acid bacteria. Carboxylate-bridged binuclear manganese centres that catalyse the disproportionation of hydrogen peroxide appear to exist in the active sites of pseudocatalase and catalase,

$$2H_2O_2 \rightarrow O_2 + 2H_2O.$$

Unlike heme catalases, these Mn-containing catalases are only weakly inhibited by cyanide or azide.

A low-resolution X-ray crystallographic analysis of the latter enzyme demonstrated that two manganese ions are located at a distance of ~ 3.6 Å, and an EPR study indicated that the binuclear core can exist in three oxidation levels, $Mn^{II}Mn^{II}$, $Mn^{II}Mn^{III}$ and $Mn^{III}Mn^{IV}$. These enzymes have been proposed to have manganese analogues of the hemerythrin active site based on spectroscopic similarities of the enzymes with corresponding dimanganese models.

Recently, a new type of ribonucleotide reductase that did not require coenzyme-B12 or iron for activity was discovered; this enzyme requires manganese instead. The electronic spectrum of the B2 subunit closely resembles

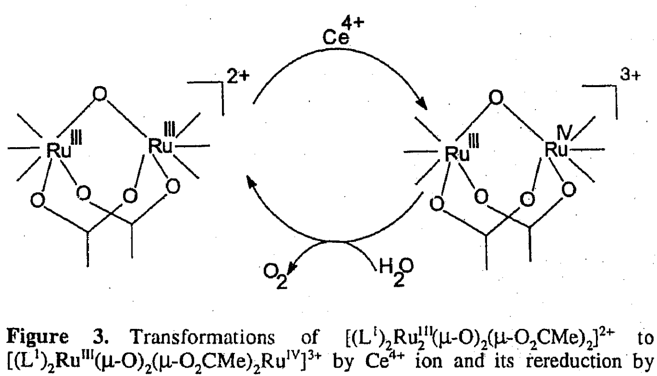


Figure 3. Transformations of $[(L^1)_2Ru_2^{III}(\mu\text{-}O)_2(\mu\text{-}O_2CMe)_2]^{2+}$ to $[(L^1)_2Ru^{III}(\mu\text{-}O)_2(\mu\text{-}O_2CMe)_2Ru^{IV}]^{3+}$ by Ce^{4+} ion and its rereduction by H_2O in acetonitrile.

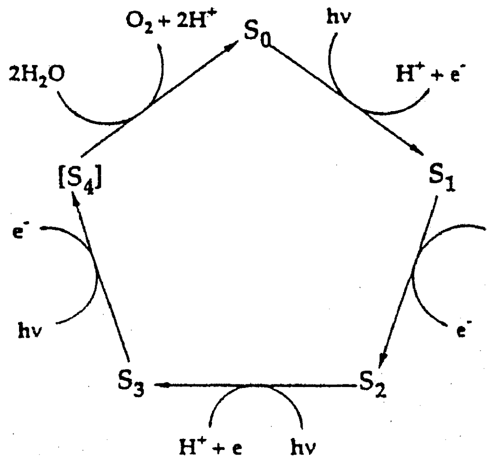


Figure 4. The S-states of the mechanism of O_2 evolution in PS II.

those of Mn-catalase and synthetic tribridged complexes with $[Mn_2^{III}(\mu\text{-}O)(\mu\text{-}O_2CMe)_2]^{2+}$ cores.

High-valent manganese systems

From the foregoing discussion it is understandable that the search for accurate synthetic models is on to mimic manganese cluster of water-oxidizing complex and manganese catalase. The structural motifs of relevance to these enzymes are higher-valent dimanganese systems with oxo and carboxylato bridges. We looked for new model systems in our laboratory and hence we prepared[28] a green mixed-valent complex, $[Mn^{III}(\mu\text{-}O)_2(\mu O_2CMe)Mn^{IV}(L^1)_2][ClO_4]_2 \cdot H_2O$ (**3**). From variable-temperature (19.8–300 K) solid-state magnetic susceptibility data we found that it has a doublet ground state with $J = -144\ cm^{-1}$ (antiferromagnetically coupled Mn centres). The X-band EPR spectrum at 77 K exhibits a 16-line Mn hyperfine signal centred at $g = 2$ (Figure 6). This is representative of binuclear Mn systems including the S_2 state of the manganese centre of PS II. The observed spectrum comes exclusively from the doublet ground state of two inequivalent manganese ions. The observed spectral pattern is indicative of a situation in which one of the ions has about twice the hyperfine coupling constant of the other.

An important test for the merit of any PS II model system is the thermodynamics of its redox reaction. Cyclic voltammetric measurement shows a one-electron oxidation at 1.0 V vs SCE to the dimanganese(IV) species as well as a one-electron reduction at -0.1 V vs SCE. Interestingly, coulometric or perchloric acid oxidation generates an orange Mn_2^{IV} species.

We found that acidification with glacial acetic acid of the mixed-valent (III,IV) dimer **3** produced a reddish brown dimanganese(III) complex $[Mn_2^{III}(\mu\text{-}O)(\mu\text{-}O_2CMe)_2(L^1)_2][PF_6]_2$ **4** (ref. 29). A case for a disproportionation reaction. For **4** on repetitive scanning of the potential between the limits 0.60 and 1.50 V vs SCE, at the expense of the higher potential response a new redox wave is being formed (Figure 7). The above experiment point towards redox transformation of **4** to the one-electron oxidized form of **3** under oxidative conditions. Thus, as the potential scanning is made up to the oxidative couple of **4**, $[(L^1)_2Mn_2^{III}(\mu\text{-}O)_2(\mu\text{-}O_2CMe)]^{2+}$ is oxidized to $[(L^1)_2Mn_2^{III,IV}(\mu\text{-}O)(\mu\text{-}O_2CMe)_2]^{3+}$ which in turn transforms to $[(L^1)_2Mn^{III}(\mu\text{-}O)_2(\mu\text{-}O_2CMe)Mn^{IV}]^{3+}$.

The above results suggest that using our capping ligand L^1 it is possible to isolate manganese dimers with bis(μ-oxo) and (μ-oxo)bis(μ-acetato) motifs. These cores give rise to similar electronic structure as that of the natural systems. Redox chemistry neatly demonstrates interconversion of one core to other, having different oxidation levels. This is a finding of considerable interest in understanding the molecular events associated with water oxidation.

Binuclear copper centres in biology

Copper proteins carry out a variety of reactions that have long interested inorganic chemists. What has been of particular fascination are the structurally novel coordination sites and their associated physical and spectroscopic properties. Among the binuclear copper proteins or enzymes of relevance to this article, one of the fascinating properties is the presence of magnetically coupled binuclear copper centres. The interaction and subsequent reactivity of dioxygen (O_2) with copper ions is of great interest due to the importance of O_2-binding and/or activating proteins in biological systems[30–33].

Hemocyanin is an O_2-carrier protein found in arthropods and molluscs. The deoxy dicopper(I) form reversibly binds O_2 via its reduction to O_2^{2-}. Tyrosinase, a monooxygenase that catalyses *ortho* hydroxylation of phenols to catechols with further oxidation to quinones. A dicop-

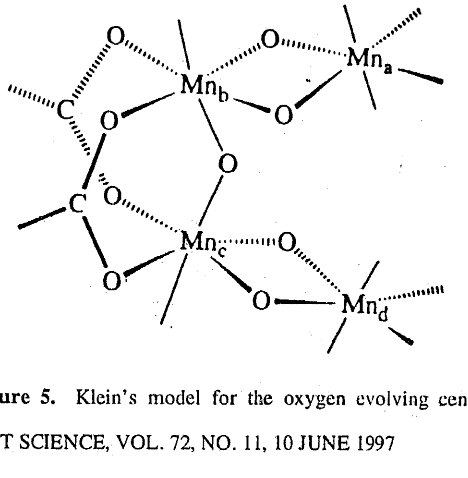


Figure 5. Klein's model for the oxygen evolving centre.

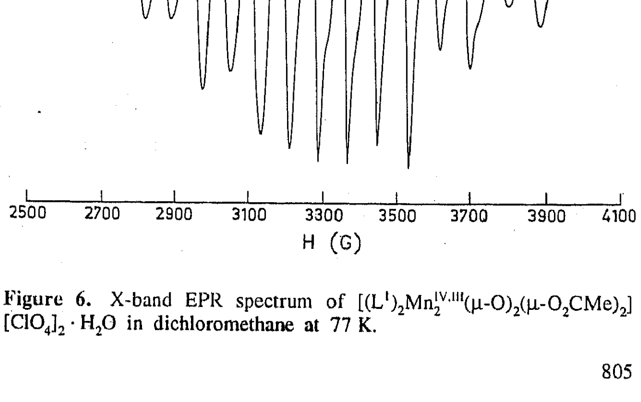


Figure 6. X-band EPR spectrum of $[(L^1)_2Mn_2^{IV,III}(\mu\text{-}O)_2(\mu\text{-}O_2CMe)_2][ClO_4]_2 \cdot H_2O$ in dichloromethane at 77 K.

per(I) centre is readily amenable to the 2e oxidation by O_2 to generate a dicopper(II) species with concomitant reduction of dioxygen to reduction of dioxygen to oxide. The resulting $Cu_2(O_2^{2-})$ species is recognized as an active intermediate or product in hemocyanin and tyrosinase.

Developments in tyrosinase model studies

Notable progress has been made to mimic tyrosinase activity using tailor-made binucleating N-donor ligands having *m*-$CH_2C_6H_4CH_2$ spacers between the coordination units. Karlin and Gultneh[30] reported the first model, consisting of a ligand that provides two tridentate *bis*[2-(2-pyridylethyl)amine] donor units to each copper ion. In the meantime a number of modified-xylyl ligand systems demonstrating aromatic ring hydroxylation have appeared in the literature. To pinpoint ligand donor atom characteristics (nature, number and their stereochemical factors etc.) necessary for bringing about aromatic ring hydroxylation in model systems, we became interested in mimicking tyrosinase-like activity using a new model system. Hence we designed a new non-Schiff base ligand (L^2), providing only two nitrogen coordination sites at each copper centre and demonstrated hydroxylation of the aromatic ring[34] (Figure 8).

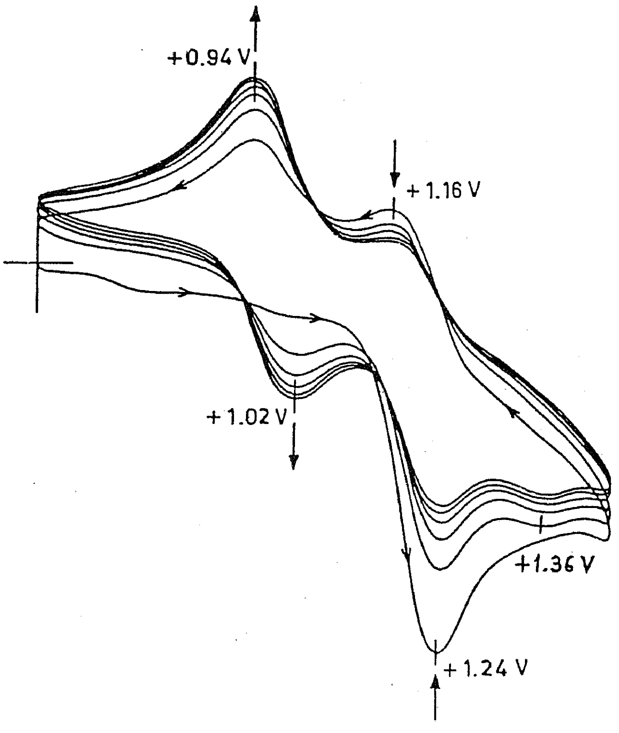


Figure 7. Repetitive scan cyclic voltammograms of [(L^1)$_2$Mn^{III}(μ-O)(μ-O_2CMe)$_2$][ClO$_4$]$_2$ · H_2O in acetonitrile at a platinum electrode; supporting electrolyte, tetra-*n*-butylammonium perchlorate.

The X-ray structure of the hydroxylated product provides, within the non-Schiff base family, the first example of a μ-phenolato and μ-hydroxo bridged copper(II) complex (Figure 9). This is a finding of considerable interest in the context of biomimetic studies aimed at the functional properties of tyrosinase.

Concluding remarks

A few potential synthetic models for (i) accurate electronic structural model for binuclear oxo-bridged iron centre of met form of hemerythrin and its ruthenium analogue, (ii) binuclear oxo-bridged manganese centres of oxygen-evolving complex of PS II (S_2 state) and catalase and (iii) binuclear copper centre of tyrosinase have been synthesized and thoroughly characterized. These models have provided electronic structural and functional properties of the aforesaid metalloproteins and enzymes. Most noteworthy results are (i) use of unsymmetrical tridentate nitrogen-donor capping ligand L^1 and a new *m*-xylyl ligand system L^2 in these models, (ii) bridge-exchange reaction on iron(III) dimer, (iii) demonstration of water oxidation with the one-electron oxidized form ruthenium analogue, (iv) redox transformations of $[Mn_2^{III}(\mu\text{-O})(\mu\text{-}O_2CMe)_2]^{2+}$ to $[Mn^{III}(\mu\text{-O})_2(\mu\text{-}O_2CMe)Mn^{IV}]^{2+}$ under oxidative conditions and (v) aromatic hydroxylation with a new *m*-xylyl ligand system. The results on the diiron(III) system provided us with the impetus to carry out research in this fascinating field. Our results on dimanganese systems demonstrated that redox-driven structural changes play an important role at the active site of PS II to increase the oxidation levels of manganese centres to oxidize water and hence

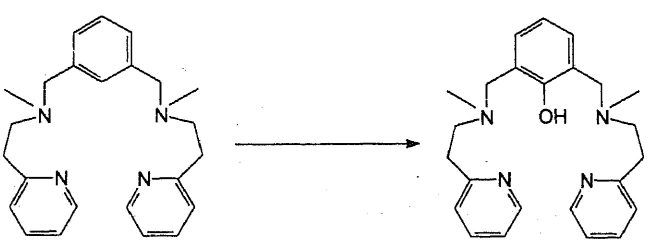


Figure 8. Demonstration of aromatic hydroxylation with our new ligand system.

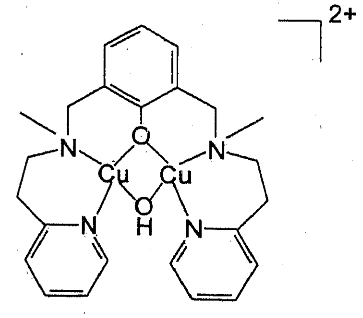


Figure 9. X-ray structure of the μ-phenoxo μ-hydroxo bridged dicopper(II) complex.

to liberate molecular oxygen. Our results on a new tyrosinase model is the demonstration of functional properties of this monooxygenase. It is obvious that there is a need and scope for understanding, at the molecular level, the fascinating properties of oxo-bridged binuclear iron, manganese and copper centres, which are still ill-understood. These include: (i) to model the functional properties of hemerythrin; at least the magnetic properties of oxyhemerythrin (hydrogen-bonded oxo-bridge), (ii) functional properties of PS II and (iii) to identify the electronic and steric factors responsible for bringing about aromatic ring hydroxylation (tyrosinase functional mimicking), etc. Such studies are in progress in our laboratory.

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New photonucleases based on tetrapyrrole, polypyridine and diazo-arene chromophores

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Binding and cleavage of DNA by new, rationally-designed photonucleases based on porphyrin, metallo-polypyridyl and diazo-arene chromophores are described in light of their application as photosensitizers in the photodynamic therapy of tumours and as structural/spectroscopic probes for DNA.

CURRENTLY considerable attention is being focused on the design of small molecules that can bind and cleave DNA. These molecules, commonly referred to as artificial nucleases, have numerous biochemical and biomedical applications. While most of the hither to reported DNA-cleaving agents have been activated thermally, in recent years, there is an increasing emphasis on photoactivated cleavage agents, because this methodology possesses significant practical advantages. In particular, photonucleases can be triggered by exposure to light; light is an attractive 'co-factor' since it is easy to manipulate. This article summarizes some of our recent results on the design, DNA-binding and DNA-cleavage of new, rationally-designed photonucleases based on porphyrin, metallo-polypyridyl and diazo-arene chromophores. Our interest in the DNA-binding and DNA-photocleavage chemistry of these chromophores is mainly related to the use of porphyrins as photosensitizers in the photodynamic therapy of malignant tumours and metallo-intercalators as structural/spectroscopic probes and site-specific cleavage agents for DNA.

DNA photocleavage by 'porphyrin-intercalator' and 'porphyrin-chemotherapeutic drug' conjugates – relevance to PDT

Photodynamic therapy (PDT), involving the action of light on a photosensitizer to generate singlet oxygen ($^1O_2$, or other reactive oxygen species) that ultimately leads to tumour necrosis, is currently being explored as an alternative modality to radio- and chemotherapy for the treatment of cancer (Figure 1)[1–3]. DNA is considered to be one among the more susceptible intracellular sites of photodamage during such a photodynamic action.

Ideally, the treatment of tumours by PDT requires a photosensitizer which is a single, nontoxic, stable compound of known chemical structure that can be retained with a high degree of selectivity in malignant tumours. In addition, from the photochemical point of view, an ideal photosensitizer should be able to absorb light in the tissue-transparent region (ca. 600 to 800 nm) and photo-generate high yields of $^1O_2$. Porphyrins, by virtue of their robust, 18 π-electron system and their rich redox and photophysical activity as well as biocompatibility, fulfill most of these criteria. Indeed, haematoporphyrin derivative (HpD or Photofrin II®) is currently being marketed as an anti-cancer drug[4]. However, the uncertainty about the chemical structure and mechanism of action of HpD and, moreover, its ability to absorb only weakly in the red region of the visible spectrum necessitated the development of other photosensitizers for use in PDT. A plethora of new potential PDT agents based on porphyrin and related macrocycles has been reported recently[5,6]. Many of these second generation photosensitizers, although absorb strongly in the 600–800 nm region and generate $^1O_2$ in good-to-moderate yields, suffer severely from their inability to accumulate in tumours.

One possible way to circumvent the problem of low accumulation of a given porphyrin-based drug in the cells is to equip the drug with an intracellular recognition element, e.g. a DNA/membrane-binding agent. This

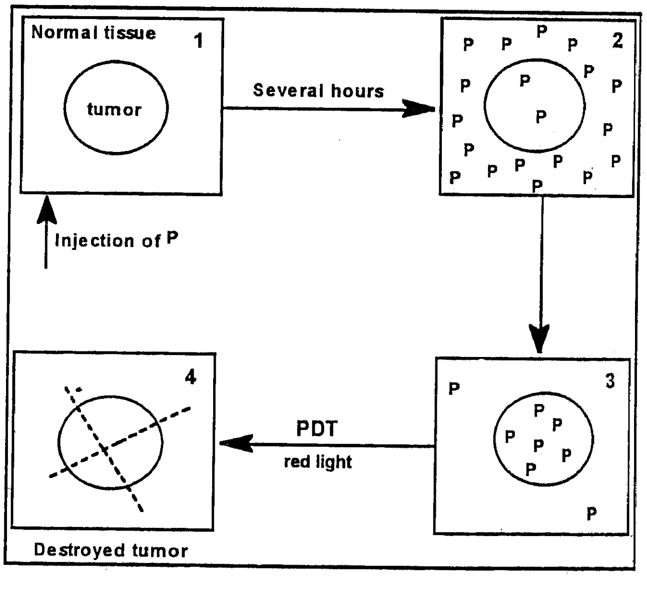

Figure 1. Schematic diagram showing the principles involved in photodynamic therapy.

approach has the potential to deliver a better tumour-targeting device, as the appended intracellular recognition element can, in principle, enhance the cellular uptake of the drug. Porphyrin conjugates endowed with oligonucleotide, monoclonal antibody, ellipticene, or other intracellular recognition elements have been reported, but the majority of these conjugates have been shown to effect the DNA cleavage only via chemical means and, very little effort seems to have been done to induce the photocleavage of DNA utilizing these modified photosensitizers[7–10]. This has prompted us to investigate on the photonuclease activity of rationally-designed 'porphyrin-intercalator' conjugates in light of their potential application to PDT.

In addition to the DNA intercalators, clinically used anti-cancer drugs can also be grafted onto the porphyrin photosensitizers and the so-derived 'combination drugs' are expected to be beneficial in the co-lateral application of chemo- and photo-therapy. Specifically, such 'porphyrin-chemotherapeutic drug' conjugates, while permitting the chemotherapeutic-drug-mediated conventional therapy to be carried on both in the presence (light-on) and absence (light-off) of light, enable the photodynamic therapeutic condition to prevail upon irradiation into the porphyrin absorption bands with the visible light. Synergistic effects toward the tumour eradication are thus expected to predominate during the irradiation period. In addition, these new hybrids can also refine the ability of the porphyrin to home the cancerous cell/tissue by virtue of its conjugation to the chemotherapeutic drug which, on the other hand, would usually possess an inherent ability to localize in the cancerous tissue.

The above concepts have been actualized by us recently and, a few recent examples of 'porphyrin-intercalator' and 'porphyrin-chemotherapeutic drug' conjugates that are capable of photocleaving DNA will be discussed in this article. More than two dozens of new hybrids in which a porphyrin is linked, via, varying lengths and types of covalent bridges (spacers or linkers), to either a DNA intercalator (e.g. acridone, phenothiazine or acridine) or a chemotherapeutic drug (e.g. chlorambucil – a DNA cross-linking 'mustard', currently marketed as an anti-leukaemic drug) were synthesized and their photonuclease activity investigated[11–13]. The structures of a few such hybrids are shown in Figure 2.

As seen from Figure 2, the porphyrin unit has been covalently linked to the intercalators or the chemotherapeutic drug *only* at those sites on them that are found to be not absolutely essential for the intercalating/antitumour activity. Similarly, enough care has been taken to ensure that the spectral and photophysical properties of the porphyrins that are necessary for their photodynamic activity remain essentially unchanged even upon linking them to the intercalator/chemotherapeutic drug subunits. Thus, the intrinsic chemical, photochemical and, consequently, the biological activities of the constituent components were expected to be unaltered in these new hybrids. This is indeed the case as evidenced by the UV-Vis, ^{1}H NMR and electrochemical data which suggest that there is minimal ground state interaction between the porphyrin and the intercalator/chemotherapeutic drug π-rings in these new conjugates. In addition, the fluorescence and singlet oxygen quantum yields of the porphyrin parts of these new hybrids are also in the same range as those of reference porphyrin **5a**.

While all of the hybrid porphyrins investigated in this study did not affect the DNA (supercoiled pBR 322) cleavage in the absence of irradiation, the reference compounds, which either lack the intracellular recognition element (ca. **5a** or an analogue) or the porphyrin (i.e. individual intercalators or chlorambucil) showed no nuclease activity both in the presence and absence of light. On the other hand, the hybrid compounds **1a–e**, **3a–d** and **4a–f** all affected relaxation of the supercoiled form I to generate open circular form II when irradiated by visible light ($\tau > 400$ nm). Interestingly, the cationic porphyrins **3h–j** displayed DNAse activity. 'Inhibitor' studies revealed that all of these photocleavage reactions predominantly proceed through a $^1O_2$-based mechanism.

Among the many interesting features noticed during the photocleavage studies with compounds **1–4**, the one that is concerned with the role of 'linker' moiety connecting the photosensitizer and the intracellular recognition element merits further discussion. This aspect can be illustrated by considering the example of porphyrin-phenothiazine conjugates **3a–j** (ref. 13). The observed highest cleavage efficiency for **3a** ($n = 3$) compared to the cleavage ability of any other porphyrin in that series and the DNAse activity of the dicationic species **3h–j** are revealing in this regard. While the shorter chain length between the porphyrin and the intracellular recognition element in **3a** is responsible for a better cleavage ability of this hybrid photosensitizer, the DNAase activity observed for compounds **3h–j** is possibly due to their hydrophillic nature and additional involvement of Coulombic interactions between the quaternized piperidine and the phosphate backbone of DNA. In this regard, it can be noted that $^1O_2$ has to diffuse within its lifetime to effect the DNA cleavage and that this process is more facile in the case of hybrids **3a** and **3h–j** where the distance between the porphyrin moiety (the $^1O_2$ generator) and the appended DNA intercalator is short (Figure 3).

Thus, besides a strong DNA-binding by the intercalator and the spacer, a close proximity of the porphyrin moiety and the appended intracellular recognition element play a crucial role in the photocleavage efficiency of these new PDT agents.

The DNA photonicking efficacy exhibited by the

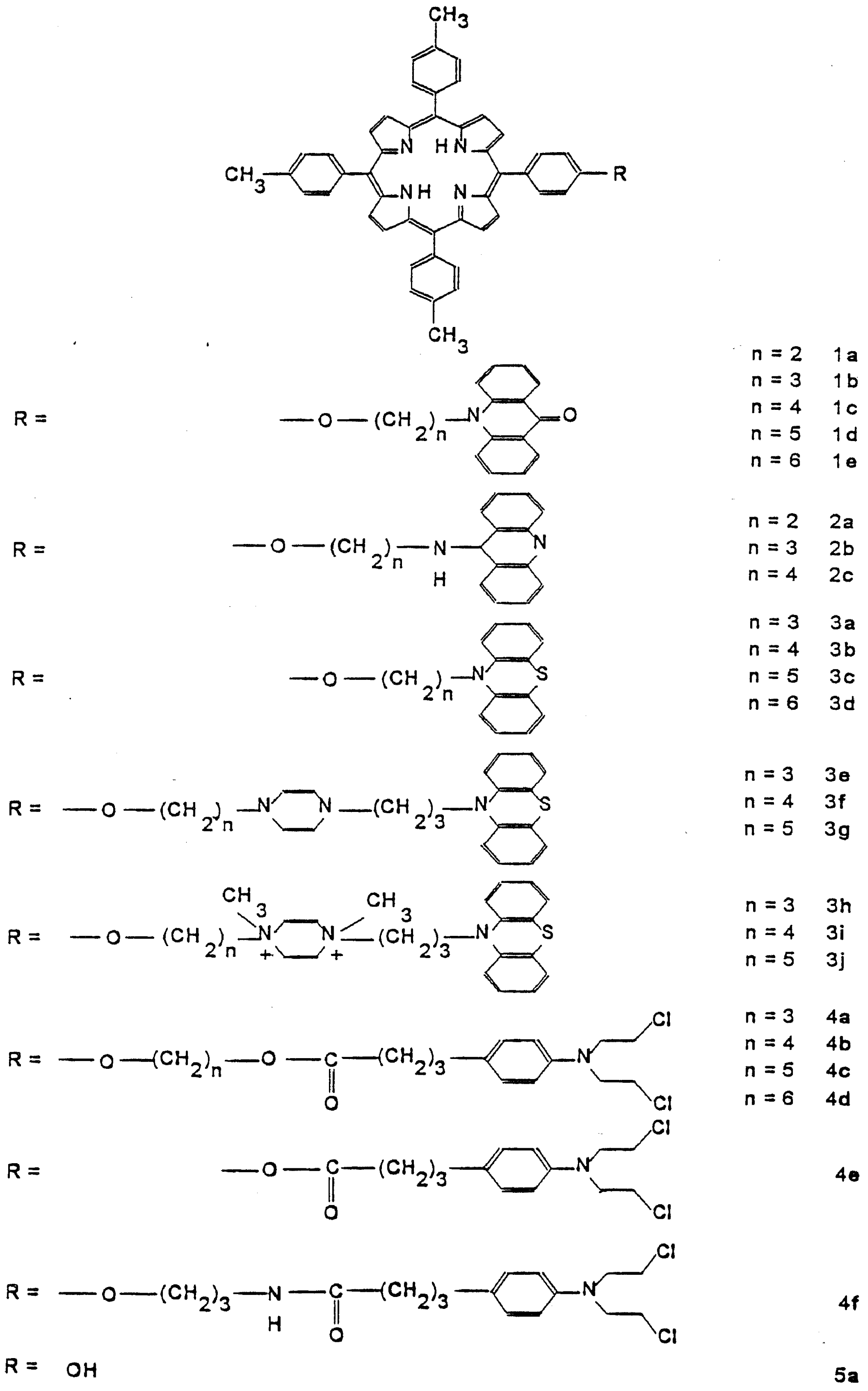

Figure 2. 'Porphyrin-intracellular recognition element' conjugates – new generation photodynamic therapy agents.

porphyrin-chlorambucil (P-Chl) hybrids (**4a–f**) was observed to be the best among all of hybrids **1–4**. This is illustrated in Figure 4 which shows that irradiation of P-Chl hybrid **4a** results in an efficient, near quantitative conversion of the supercoiled form I DNA to form II.

In addition to this observation, the fact that P-Chl hybrids can also act as chemotherapeutic drugs even in the absence of light (compounds **4a–f** were seen to convert the single-stranded DNA to the double-stranded variety in dark) prompted us to amplify the theme of what can be nick-named as the 'double war-head' approach and to construct new, more potent PDT agents. Accordingly, a new series of PDT agents in which a porphyrin is linked either to one (P-col-Chl) or two (P-col-Chl$_2$) chlorambucil subunits, via, a cholic acid (col) were designed[14]. The cholic acid 'platform' had been specifically employed for the construction of these newer hybrids owing to its ability in recognizing and localizing in the liver cells. As expected, P-col-Chl$_2$ was found to be much more active in its DNA cleavage ability in comparison with either P-col-Chl or compounds **4a–f**.

All these results indicate that the combination of a intercalator/chemotherapeutic drug with a photosensitizer enhances DNA cleavage proclivity under the influence of visible light. The cellular level *in vitro* photodynamic efficiency of these new hybrids also suggests the same; preliminary data indicate that the P-Chl diads and triads effectively photokill the TF-1 leukaemic cancer cells when irradiated by visible light[15]. In this regard, it may be noted that, notwithstanding the recent reports which suggest that the current clinical PDT agents target the cell membrane, it is essential to examine the effect of photoirradiation on DNA in the presence of porphyrin conjugates such as those discussed here. These studies are also useful in the development of new and more efficient PDT agents.

DNA-binding and photocleavage by metallo-intercalators

Metal complexes are particularly attractive species for developing new diagnostic and therapeutic agents that can recognize and cleave DNA[16,17]. The ligands or the metal in these complexes can be varied in an easily controlled manner to facilitate an individual application. We have been interested to know the effect of variation of the metal ion and also the ligand in complexes containing strongly intercalating and photo-active ligands on their ability to bind and cleave DNA. Dipyrido-(3,2-a:2′,3′-c)-phenazine (dppz) seemed to be an ideal ligand to start with because (i) it is a near-planar, heteroaromatic entity and hence, can intercalate between the base-pairs on DNA, (ii) its complexes absorb in the visible region and (iii) it can be easily modified to suit various applications[18,19]. In a separate study that is unrelated to PDT, we have investigated the DNA-binding and photocleavage by a series of new [M(phen)$_2$(LL)]$^{n+}$ type (M = Co(III), Ni(II), or Ru(II), phen = 1,10- phenanthroline, LL = dppz or modified dppz and $n = 3$ or 2) metallo-intercalators by using absorption and emission

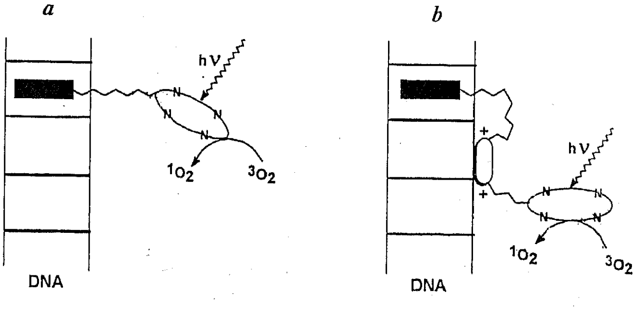

Figure 3. Possible modes of DNA-binding and DNA photocleavage by porphyrin-phenothiazine hybrids (*a*) **3a** and (*b*) **3h–j**.

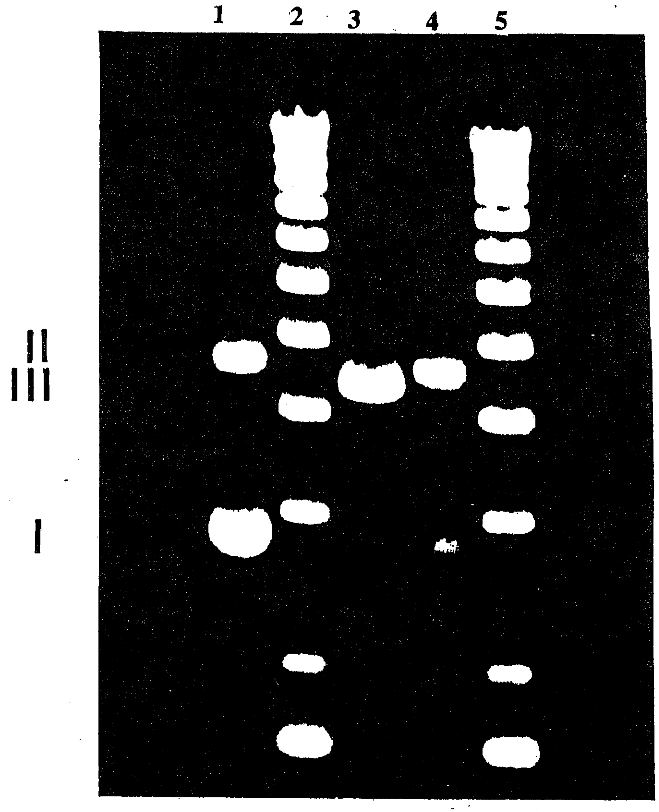

Figure 4. Light-induced nuclease activity of porphyrin-chlorambucil hybrid **4a**. (From left to right): Lane 1: untreated pBR 322 DNA; Lane 2: DNA marker; Lane 3: pBR 322 digested with *hin*dIII; Lane 4: pBR 322 irradiated in the presence of **4a** ($\lambda > 400$ nm, 150 W Xe-arc lamp, 1 mW/cm^2, $t = 10$ min); Lane 5: DNA marker. Supercoiled, relaxed-circular and linearized DNA have been marked as I, II and III, respectively on the gel. Gel electrophoresis experiments were carried out as described in refs 11–14 and 20, 21.

titration, thermal denaturation, gel electrophoresis including the topoisomerase assay and electrochemical methods.

Initially, effects of changing the metal ion in the mixed-ligand dppz complexes on their DNA-binding and photocleavage characteristics were investigated. Two new mixed-ligand complexes, containing either cobalt(III) ($[Co(phen)_2(dppz)]^{3+}$, **6**) or nickel(II) ($[Ni(phen)_2(dppz)]^{2+}$, **7**) as the central metal ion, were seen to be avid binders of calf thymus (CT) DNA[20]. Results of various spectroscopic, electrochemical and biochemical investigations carried out with these complexes revealed that the dipyridophenazine ligand on them is engaged in the intercalative interaction with DNA and that the intrinsic binding constant, K_b, is as high as $(9 \pm 2) \times 10^5 \, M^{-1}$ for both the complexes. Thus, although these two complexes provided a good opportunity to compare directly the binding of isosteric intercalating species of +2 and +3 charge to DNA, such a comparison could not be made. On the other hand, while the cobalt(III) complex was found to affect photocleavage of the supercoiled pBR 322 DNA via a $OH^{\bullet}$-based mechanism, as evidenced by 'inhibitor' and 'spin-trapping/ESR' studies, the nickel(II) complex (a d^8 system), being paramagnetic, was ineffective under similar experimental conditions. Thus, these results, while underscoring the importance of dppz in the DNA-binding, also demonstrate that substitution by different metal ions can bring about subtle modulation in the properties and, consequently, in the DNA interaction of this new class of mixed-ligand complexes containing the versatile dipyridophenazine ligand.

During our continued investigations on the dppz-based complexes, it occurred to us that further derivatization of this ligand with suitable electron withdrawing/donating group(s) might not only accentuate DNA-binding and photocleavage efficiencies of the ensuing complexes but also serve to explore other interesting functional aspects associated therein. A series of new, mixed-ligand ruthenium(II) complexes, incorporating modified dppz ligands, have been designed recently. The DNA-binding and photocleavage abilities as well as redox and luminescence properties of two representative complexes will be discussed here.

The mixed-ligand ruthenium(II) complex **8**, incorporating a quinone-fused dipyridophenazine ligand [10,11-(1, 4-napthalenedione) dipyrido (3, 2,-a:2′, 3′-c)phenazine, qdppz] was synthesized first[21]. The choice of quinone moiety for modifying dppz is owing to the known DNA-binding ability and the rich redox chemistry of this ubiquitous electron-deficient functional group[22,23]. Chemical or electrochemical reduction of **8** lead to the generation of **9** – a complex containing the hydroquinone form of qdppz (Figure 5).

Complex **8** is an avid binder of CT DNA ($K_b \geq 10^6 \, M^{-1}$) due to a strong intercalation by the ruthenium-bound qdppz whereas, the reduced complex **9** shows only a moderate binding ($K_b = (2 \pm 0.3) \times 10^4 \, M^{-1}$). The DNA photocleavage efficiencies of these complexes also follow a similar trend in that the MLCT excited state of **8** is more effective than that of **9** in cleaving the supercoiled plasmid pBR 322 DNA ($\lambda_{exec} = 440 \pm 5$ nm). While irradiation into the MLCT band of **8** can generate a species containing oxidized ruthenium and reduced qdppz (semiquinone), direct excitation of the bound-qdppz is expected to provide the triplet quinone. Both these quinone-based, transient species are known to be potent DNA-cleaving agents capable of reacting with the duplex via various mechanisms, including hydrogen abstraction, electron transfer, etc.[22]. Finally, by the combined application of exhaustive bulk-electrolysis and steady state fluorescence spectroscopic methods, it was demonstrated that the '$2e^-/2H^+$' redox couple **8/9** represents a molecular light-switching device, displaying interconversion between the non-luminescent 'quinone' and luminescent 'hydroquinone' states in aqueous CH_3CN solutions (Figure 5)[21]. Thus, these complexes are useful not only in the design of photonucleases but also in the development of molecule-based, electronic devices.

Tris- and mixed-ligand ruthenium(II) complexes, which incorporate another modified dppz ligand dicnq [10, 11-dicyanodipyridoquinoxaline], were investigated next; complex **10** is a typical example (Figure 6)[24].

It was observed that these complexes are also avid binders of DNA by the intercalative mode and, in addition, that they photocleave DNA when irradiated into their MLCT absorption envelope. The most interesting aspect of these compounds is, however, their 'light-switching' ability in the presence of DNA. For example, while complex **10** is nearly non-luminescent in aqueous buffered solutions, addition of increasing amounts of DNA to the aqueous buffered solutions, containing this complex, was seen to result in the luminescence enhancement to the tune of ca. 15–20 times (Figure 6). Thus, this complex is an efficient 'luminescence reporter' of DNA.

Carbene-mediated DNA photocleavage

In a majority of earlier studies with photonucleases, photogenerated ion, radical or oxygen-centered reactive species have been proposed to be responsible for cleaving DNA. However, carbenes – a class of unstable and highly reactive organic species, which are known to be generated upon photoirradiation of arene diazo compounds and to be reactive towards a wide variety of organic and bioorganic substrates including proteins – seem to have never been tested for their ability to cleave DNA[25]. In a more recent study, we have been able to show, for the first time, that photolysis of simple, readily available arene diazo compounds leads to the cleavage of DNA via carbene-mediated pathway[26].

9-Diazoanthorne (**11**), a carbene-precursor capable of absorbing the visible light (λ_{max}(DMF) = 431 nm), has been chosen here for the demonstration of carbene-mediated DNA photocleavage not only because of its well-known photochemical reactivity but also for its planar, aromatic structure which assists in the intercalative binding with DNA. Irradiation of supercoiled pBR 322 DNA by light of $\lambda > 400$ nm in the presence of this diazo-arene resulted in a facile generation of the relaxed circular form and, careful experiments with various 'inhibitors' established the participation of carbene species in this photoconversion. Specifically, while D_2O, mannitol, dimethyl sulphoxide and super-oxide dismutase did not affect the DNA-nicking efficiencies of **11**, suggesting that none of the reactive oxygen species ($^1O_2$ $OH^{\bullet}$ or $O_2^{\overline{\bullet}}$) play a role in the cleavage mechanism, a 90–60% inhibition exhibited by 2-propanol, triphenyl phosphine and cumene collectively provided evidence for the participation of carbenes in the observed DNA cleavage by this reactive diazo compound.

Concluding remarks

In summary, we have attempted to show here that it is

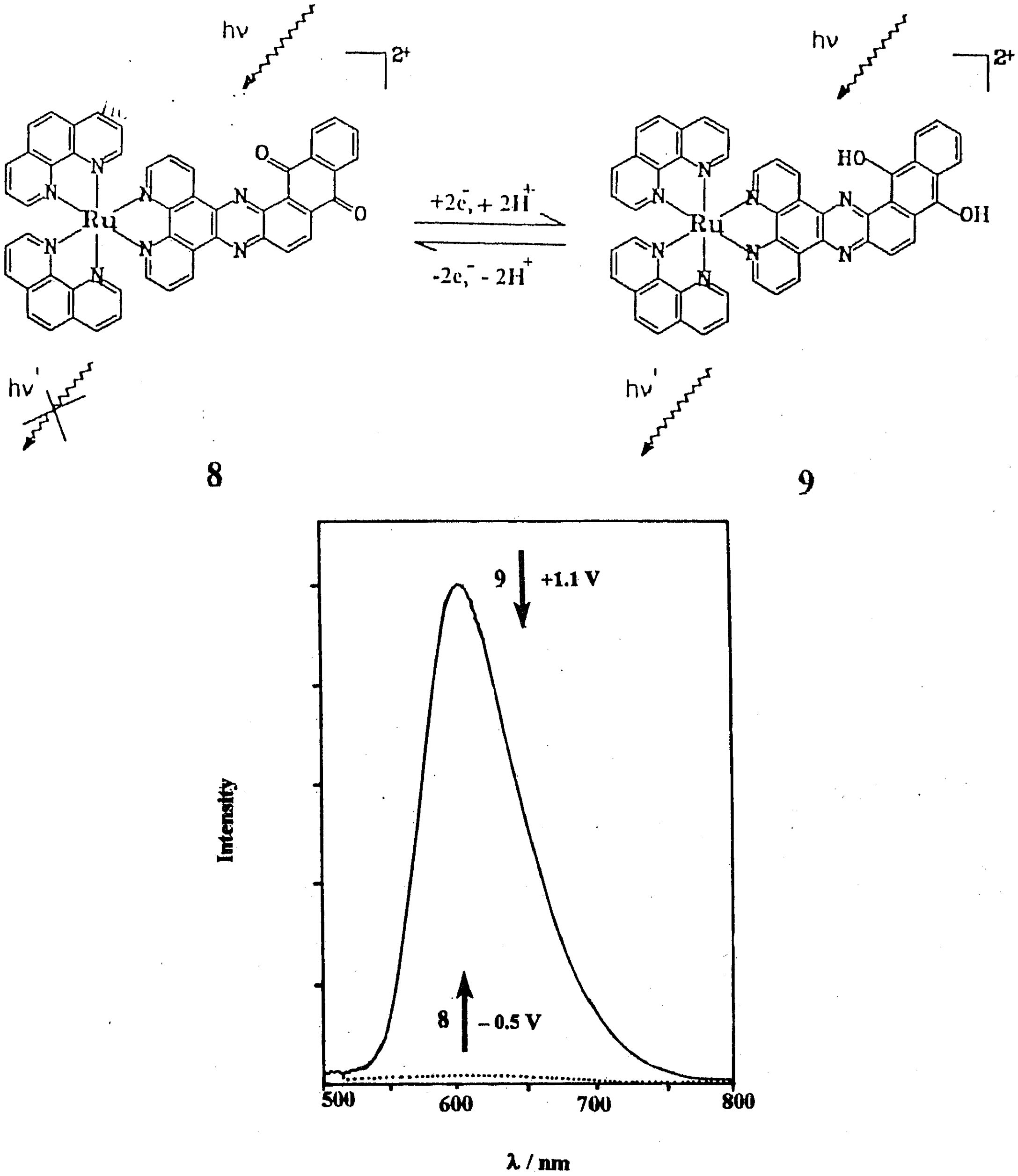

Figure 5. An 'electro-photo switch': Luminescence spectra (CH_3CN/5% H_2O, 0.1 M $TBAPF_6$, λ_{exc} = 440 nm) of **8** and **9** as obtained by exhaustive electrolyses at the indicated potentials in each case. The arrows refer to the reversible changes observed upon electrochemical interconversion of these complexes.

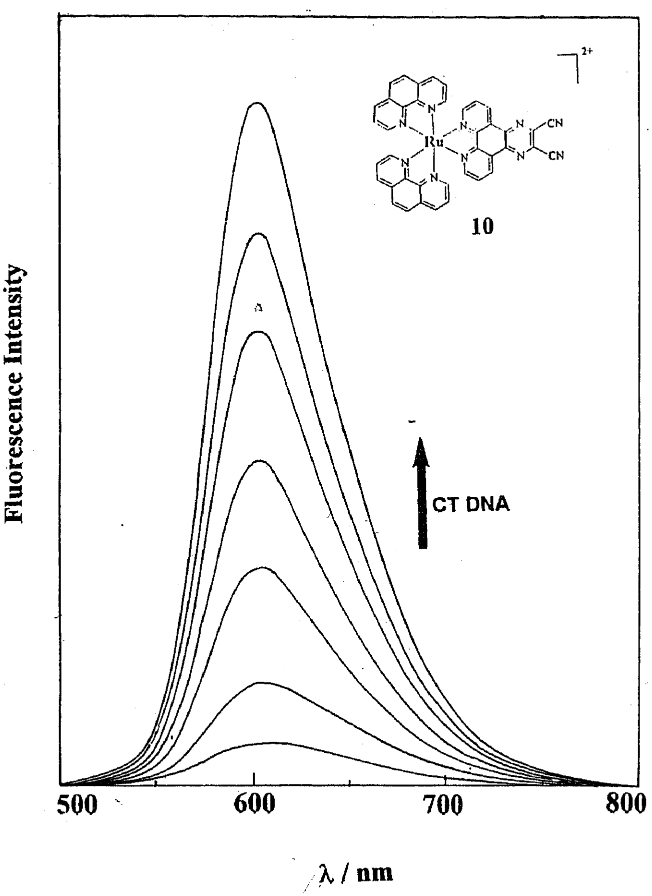

Figure 6. Luminescence enhancement observed during the addition of increasing concentrations (0–600 mm) of CT DNA to a buffered solution containing complex **10** (10 mM) – a 'luminescence reporter' of DNA. ($\lambda_{exc} = 440$ nm).

possible to accomplish potent photonucleases by suitably modifying the structures of visible-light-absorbing chromophores. A highlight of this approach is that the chromophores either themselves can intercalate between the base-pairs of DNA (e.g. diazo arenes) or can employ other structural components grafted on them to strongly bind to the duplex by noncovalent (intercalation) and covalent (alkylation) interactions. The principal theme, common to these new photonucleases, is that they all absorb light in the 400–650 nm region, generate cytotoxic species such as reactive oxygen intermediates ($^1O_2$ or $OH^\bullet$), radical or carbene species and lead to DNA cleavage. The photonucleases described here also have several practical applications. The 'dual action' theme, as exemplified in studies with the porphyrin-chlorambucil conjugates, can be perceived to have immense application in the 'combination therapy' (i.e. chemotherapy + phototherapy) against cancer. The 'light-switching' ability of complex **10** in the presence of DNA permits the use of this complex as a 'luminescence reporter' of DNA.

Finally, DNA-binding and photo-cleavage by complexes **8** and **9** together with the finding that this redox couple represents an 'electro-photoswitch' testify to the suitability of these complexes in the design of both photonucleases and molecule-based, opto-electronic devices. Currently, we are engaged in examining the site-specificity involved in the DNA-binding and DNA photocleavage of these novel photonucleases.

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Antibody responses revisited

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Humoral responses are complicated, multi-step processes involving initial antigen driven activation of B cells, selection, amplification and finally differentiation into either antibody secreting plasma cells or memory B cells. While the initial activation is of low-stringency resulting in a heterogenous population of antigen-activated B cells, selection criteria are subsequently enforced which limit the number of B cells that persist during maturation of the antibody response. This article discusses our attempts at using designed antigens to unravel the processes governing B cells selection.

THE immune system consists of a wide range of distinct cell types, each with important roles to play. The lymphocytes occupy central stage as they are the cells that determine the specificity of immunity, and it is their response that orchestrates the effector arms of the immune system. Individual lymphocytes are specialized in that they are committed to respond to a limited set of structurally related antigens. This commitment, which predates the first contact of the immune system with a given antigen, is expressed by the presence on the lymphocyte's surface membrane of receptors specific for determinants (epitopes) on the antigen. Each lymphocyte possesses a population of receptors, all of which have identical combining sites. One set, or clone, of lymphocyte differs from another clone in the structure of the combining region of its receptors and thus differs in the epitopes, expressed on immunogenic substances, that it can recognize. The ability of an organism to respond to virtually any 'non self' antigen is achieved by the existence of a very large number of different clones of lymphocytes, each bearing receptors for a distinct epitope[1]. As a consequence, lymphocytes are an enormously heterogeneous group of cells. For example, the number of distinct combining sites on lymphocyte receptors of an adult human far exceeds 10^9.

Two broad classes of lymphocytes are recognized: the B lymphocytes, which are the precursors of antibody-secreting cells, and the T lymphocytes. T lymphocytes play an important role in regulating antibody production by the B lymphocytes as a consequence of a direct, cognate interaction between these two classes of lymphocytes. This interaction is, however, highly specific in that both cells must have specificities for determinants borne by the same antigen.

An encounter between antigen and the host preimmune B cell repertoire (i.e. the B cell pool prior to contact with an antigen) results in the activation of that subset bearing appropriate surface immunoglobulin (sIg) receptors. The principal outcome of such a recognition is the induction of antigen-specific IgM antibodies. Subsequent developments include antibody isotype switch (usually predominantly to IgG) and B cell differentiation into either memory or antibody-secreting plasma cells. In T-dependent responses, progression of antigen-activated B cells through each of these stages is driven by antigen-specific activated T helper cells. B cells bind antigen through their sIg receptors, which is then followed by endocytosis, antigen processing and presentation of appropriate fragments in the context of MHC Class II molecules. Such antigen-presenting B cells then engage relevant T helper cells in a cognate interaction, one consequence of which is B cell proliferation and differentiation into antibody-secreting plasmacytes[2].

Problem of selective and hierarchical immunodominance

Unequal antibody responses to the spectrum of epitopes displayed by a multideterminant antigen have remained a long-standing paradox. The seemingly large preimmune B cell repertoire endows a given mammalian host with the potential to recognize a virtually limitless array of antigenic determinants. This along with the fact that B cells generally recognize protein antigens in their native form has led to the dogma that the entire accessible surface of a protein represents an antigenic continuum[3]. While this dogma is a logical derivative of our understanding of the mechanics of humoral (B cell) responses it, nevertheless, contrasts with experimental observations. With a variety of protein antigens[4,5], it has been the common finding that only a fraction of the B cell epitopes presented is actually successful at eliciting antibodies. While such 'selective' immunodominance clearly represents one aspect of the problem, there is yet another which is characterized by unequal levels of antibodies obtained against even those that are recognized in the humoral response. Thus the collective phenomenon of selective and hierarchical immunodominance implicates the involvement of parameters in addition to that of surface accessibility of the determinant alone in the induction and progression of humoral responses to polypeptide antigens.

The problem as stated above achieves practical significance in the context of design of new generation vaccines. With rapid advances in immunology it is increasingly more feasible to precisely identify neutralization epitopes, i.e. those epitopes capable of inducing neutralizing antibodies, on pathogen-derived proteins. This ability is also concurrently paving the way for the eventual development of 'minimalistic' vaccines that incorporate only the relevant epitopes rather than the whole pathogen or its antigenic components. Towards this end a variety of modalities are being explored[6] that include, among others, incorporation of epitopes into live vector vehicles[7-10], chemical linking of epitopes[11,12], co-oligomerization of self-assembling peptides[13], and collinear assembly of polyvalent immunogens either by chemical synthesis[14,15] or by rDNA routes[16]. Regardless of the particular route one adopts, an important prerequisite for the eventual success of this strategy is that the included epitopes must retain their immunodominance in the new construct. It is from this standpoint that the resolution of the problem of selective and hierarchical immunodominance becomes particularly critical.

Selective immunodominance is immunologically regulated

To address some of these issues we initially expressed a designed, chemically synthesized and assembled gene coding for a hundred amino acid polypeptide, MEP-1 (ref. 16). This polypeptide included select determinants from the large protein of the surface antigens of Hepatitis B virus. A preliminary analysis indicated that MEP-1 was highly immunogenic in a variety of mouse strains and therefore held promise. However, the problem of selective and hierarchical immunodominance was also true of MEP-1. Of the three domains incorporated, only two were recognized by antibodies as revealed by peptide mapping[17]. Furthermore, of these two domains, antibodies against the amino-terminal epitope (epitope B1) predominated, accounting for over 90% of the total antibody population. On the other hand, those against the carboxy-terminal determinant (epitope B2) only constituted a minority[17]. Subsequent studies revealed that immunodominance of B1 was not a consequence of primary B cell recognition[18]. Indeed, in the first week following an immunization with MEP-1 it was the anti-B2 antibodies which predominated. However, subsequent maturation from this early primary response was accompanied by a rapid increase in anti-B1 titres, whereas the anti-B2 response was suppressed. This was found to correlate well with the ability of B cells directed against epitope B1 to productively interact with antigen-specific T helper cells relative to those against epitope B2 (ref. 18). It must be noted here that the ability of a given antigen-activated B cell to recruit help from antigen-specific T helper cells would represent the primary determinant of the extent to which this B cell would proliferate and differentiate into antibody-secreting cells. Thus the facility with which individual B cell populations activated against the various epitopes on a multideterminant antigen can access T cell help would directly correlate with the level of antibodies produced against each of those epitopes.

Further dissection of these initial results revealed an interesting paradox. Among the two initially induced polyclonal subpopulations, it was the B2-specific subset which had an affinity for antigen that was markedly higher than that against the B1. Given that the immunoglobulin (antibody) secreted by a given B cell is virtually identical to the sIg receptor on its surface, this would also imply that the B2-specific B cells would bind antigen with a higher affinity and thereby better present it to T helper cells relative to the antiB1 B cells[19]. However our observations were to the contrary. This puzzle was subsequently resolved with our finding that secreted immunoglobulins also have a critical role to play by competing with the corresponding B cells for binding to the epitope. Because of the soluble nature of secreted antibodies as opposed to the B cell surface bound receptor, they have faster on-rates for antigen binding – though affinities are identical. Thus when a given epitope induces large amounts of very high-affinity antibodies in the early primary response, it creates an equilibrium situation where the epitope on the free antigen molecules is completely saturated by the secreted antibodies (Figure 1). As a result, the antigen is now inaccessible for binding by the corresponding B cell. This renders it unable to recruit T cell help, ensuring its elimination from the response[18].

Affinity-mediated selection – the other side of the coin

These initial results clearly demonstrated that subsequent selection of B cells from the early antigen activated pool is a more complex process than hitherto anticipated. In order to delve further into this process we resorted to synthetic peptides as tools that are more amenable to both sequence and structural modifications. The parent peptide employed (peptide PS1CT3) was chimaeric peptide that included a known B cell epitope from the surface antigen of Hepatitis B virus (segment PS1) and

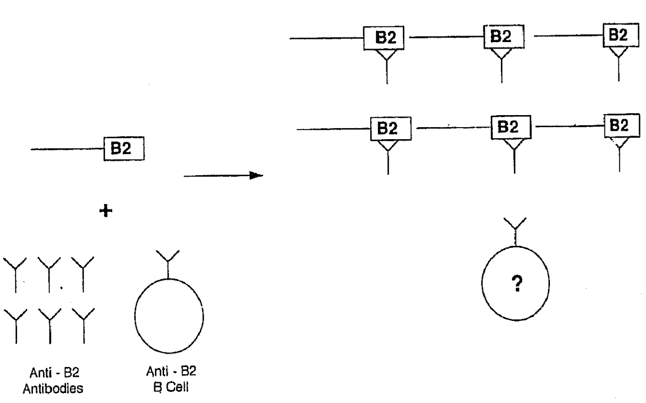

Figure 1. Secretion of epitope-specific high-affinity antibodies in the early primary humoral response down regulates further progression. Figure shows a model epitope (B2) on antigen which elicits large amounts of high-affinity antibodies in the early stages of a primary antibody response. This results in a three-way competition where the secreted antibody, by virtue of its higher on-rates, preferentially saturates the epitope. As a result, the antigen is inaccessible for B cell recognition. Further details are described in the text.

a known T cell epitope derived for the circumsporozoite protein of the malaria parasite, *Plasmodium falciparum* (segment CT3). Both epitopes were separated by a spacer of two glycine residues for reasons described earlier[20].

Immunization of mice with peptide PS1CT3 yielded an early primary IgM response that was heterogeneous with respect to fine-specificities recognized within the PS1 segment. However, subsequent progression of the humoral response saw a stringent selection for a restricted antibody specificity, exclusively directed against a tetrapeptide segment within PS1 of sequence DPAF (Figure 2). In parallel we also ascertained, from experiments in nude mice, that peptide PS1CT3 represented a T-dependent antigen during both the IgM and IgG stages of the response[21]. This peptide therefore provides a valid model for the study of B cell selection.

Our first set of experiments was to determine the quantum of T cell help required for the individual stages of primary B cell response to this peptide. While both the induction of IgM antibodies and B cell differentiation into a memory pool was readily achieved in the presence of limiting numbers of antigen-primed T cells, antibody class switch from IgM to IgG required at least an order of magnitude larger pool size of T cells. This finding allowed us to propose that induction and progression of a primary T-dependent humoral response was comprised of a single rate limiting step, in terms of T helper cell requirements, represented by the antibody isotype switch. From this we also postulated that this step would also constitute the principal 'filtering-out' point where only a fraction of the originally induced B cells are retained. This inference could subsequently be confirmed by our demonstration that selection for the 'DPAF'-specificity in the murine primary humoral response to peptide PS1CT3 also occurs concomitantly with class switch[22].

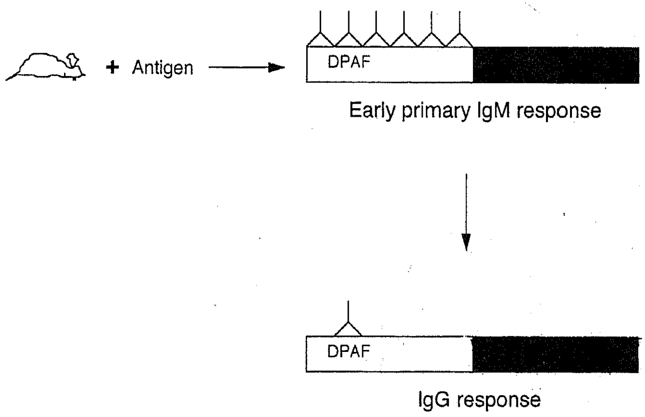

Figure 2. Maturation of a murine primary antibody response to peptide PS1CT3. Immunization of mice with peptide PS1CT3 initially induces IgM antibodies against the entire PS1 sequence (open box; sequence: HQLDPAFGANSTNPD). Maturation into an IgG response however, involves selection for antibodies only against a tetrapeptide segment, DPAF. The position of the DPAF segment within PS1 is indicated in the figure. The closed box represents the T epitope segment, CT3.

So what then are the immunological processes that guide exclusive selection for the anti-DPAF antibodies? It was this aspect that we next investigated. The circular dichroism spectrum of this peptide indicated a random distribution of conformations, suggesting that surface accessibility is unlikely to be a limiting factor. Indeed, dissection of the early primary IgM response into individual monoclonal antibodies confirmed this by revealing a composition that collectively recognized the sequence of the PS1 region. However, monoclonal antibodies derived from the late IgG stages of the response were all uniquely directed only against the DPAF sequence. Interestingly, though the IgG response was monospecific for DPAF it was nevertheless oligoclonal. Nucleotide sequencing of the antibody heavy chain variable region genes indicated at least four independent B cells contributing towards this response[21].

To examine the basis of DPAF selection, we synthesized a set of PS1CT3 analogues involving single amino acid substitutions for glycine within the DPAF segment. Of particular interest was our finding that immunization of mice with these analogues gave IgG antibody responses whose fine-specificities had now shifted to segments flanking the DPAF region. However, the affinity of these antibodies was markedly lower than that obtained against the DPAF sequence in PS1CT3. Collectively these and related studies seemed to suggest that selection for the DPAF specificity was the outcome of a competitive process where B cells directed against alternate domains were suppressed. Furthermore, based on affinities of secreted antibodies, it also seemed likely that positive selection was determined by the affinity of B cell sIg receptor for antigen. These speculations were subsequently ratified in mixing experiments where mice were immunized with an equimolar mixture of peptide PS1CT3 and one of its analogues, where antibody responses to the analogue were now completely suppressed[21].

Interestingly, our follow-up studies revealed that the driving force for competitive selection was the available pool size of antigen-specific T helper cells which, in the early stages of an immune response, is necessarily limiting. The affinity of the B cell sIg receptor for antigen merely served to define its ability – by way of antigen binding, processing and presentation – to recruit help from the limiting T cell pool and thereby denying it those with a lower affinity for antigen[21] (Figure 3).

While the results discussed above suggest that it is the limiting pool of antigen-specific T helper cells during an early stage primary antibody response that constitute the cut-off for the range of B cell affinities selected, our more recent experiments indicate that it is not an invariant barrier. Rather it represents a dynamic equilibrium between affinity of B cell for antigen and the actual pool size of antigen-specific T helper cells[22]. Thus for antigens which are more efficient at priming T cells,

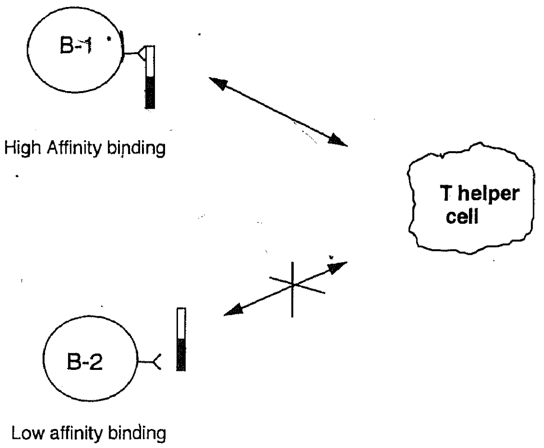

Figure 3. A limiting pool of T cell help enforces an affinity-driven competition between early activated antigen-specific B cells. The pool of antigen-specific T helper cells generated in the early stages of an immune response is insufficient to service the gamut of preimmune B cells that are activated upon initial antigen exposure. This enforces a competitive process where B cells with higher affinity for their corresponding epitope recruit help from this pool unidirectionally, leaving none available for B cells with lower affinities for their corresponding epitopes, which are then eliminated as a result.

even B cells with relatively lower affinities for antigen are selected. On the other hand, those antigens which prime T cells poorly will induce an IgG response that is far more restricted in terms of both fine-specificity and clonal diversity.

Concomitant with the above studies, we have also been examining parameters which modulate binding of antigen (through the epitope) to B cells. For this we have again used single amino acid substituted analogues of peptide PS1CT3, but with glycine substitutions performed outside of the DPAF segment though within the PS1 sequence. Such substitutions were found to alter binding properties of the resultant analogues but without affecting the fine specificity of recognition (i.e. binding through the DPAF segment was retained). These studies revealed that, in addition to affinity, the on-rate of antigen binding to receptor also influences the extent of B cell proliferation and subsequent antibody production (manuscript submitted). Thus, among a population of B cells with comparable affinities for their corresponding epitopes, those with higher epitope binding on-rates will proliferate preferentially, resulting in an antibody response that is biased in favour of such epitopes.

Summary

We believe that our investigations over the last three years have now provided us with at least a glimpse into the subtleties underlying T-dependent B cell responses. The early primary IgM response is indeed consistent with the dogma in that all accessible regions on the antigen surface are recognized[21,23]. It is the subsequent maturation from this phase where restriction is enforced. Positive selection of a particular B cell appears to be primarily determined by its ability to recruit the appropriate level of T cell help. This in turn is regulated by its affinity for epitope which indirectly defines its antigen-presenting efficacy to T helper cells. However, there is an upper threshold limit. Affinities above this threshold have a suppressive effect by ensuring elimination of the corresponding B cells as a result of competition with it for the same epitope. On the other hand, too low an affinity for epitope will also obviously not permit its recruitment by antigen. These results can therefore be summarized into an 'affinity window' hypothesis that sets upper and lower limits for affinity of preimmune B cell receptor for epitope in order to be fruitfully recruited by antigen into a humoral response. Within the limits of this window, affinity is expected to play a positive regulatory role. In addition to affinity, the extent of B cell proliferation is also modulated by the on-rate of antigen binding. This would be particularly true in situations where B cells of independent specificities have similar affinities for their corresponding epitopes. In such cases, on-rates may represent the primary determinant of which response dominates over the other. Such a hypothesis may serve towards explaining the basis of both selective and hierarchical dominance.

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Structure–function relationships and engineering of host–defense peptides

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Over the years, fatalities due to microbial infections have been reduced considerably due to the availability of a large number of potent antibiotics. The mechanism of action of these antibiotics involves either inhibition of bacterial cell wall synthesis, protein and nucleic acid biosynthesis or enzyme activities crucial to bacterial metabolism. Unfortunately, in recent years, microorganisms have developed resistance to a large number of therapeutically used antibiotics which were earlier very effective. Hence, the search for new molecules against which resistance may not develop easily is imperative. Species across the evolutionary scale from insects to mammals use peptides to combat bacteria. The endogenous antibacterial peptides exert their activity by permeabilizing the bacterial membranes. In this review, the approaches that have been employed in engineering endogenous antibacterial peptides to get molecules with improved activity, the biophysical properties that are responsible for activity and the possible therapeutic use of these class of peptides are discussed.

SPECIES right across the evolutionary scale from insects to mammals are susceptible to microbial infections[1–3]. Over the years, several antibiotics have been discovered and/or synthesized that are very effective in combating microbial infections in animals and humans[4,5]. Therapeutically used antibiotics act by either inhibiting bacterial cell wall synthesis, protein and nucleic acid biosynthesis or inhibiting enzymes whose activities are crucial to bacterial metabolism[4,5]. However, the endogenous molecular armaments that insects, amphibians and mammals use to counter micro-organisms are peptides which are composed of 20–50 amino acids[6–9]. They are synthesized as precursors by the cells' protein synthesis machinery which are processed to yield the endogenous antibacterial peptides[6–9]. Hence, they do not contain D-amino acids or other unusual amino acids like the well-studied antibiotics gramicidins[7,10,11] or alamethicin and related peptides[12]. Almost all the endogenous host–defense antibacterial peptides exert their activity by a common mechanism, i.e. by permeabilizing membranes[6–9] unlike therapeutically used antibiotics. A large number of them also act only on bacteria and not on eukaryotic cells. In recent years, resistance to therapeutically used antibiotics[13–16] has emerged as a very serious problem in clinical medicine[4,5,17] making newer, novel approaches to overcoming resistance imperative. Since species separated by millions of years in evolution use membrane-active peptides to kill bacteria, it is likely that resistance may not develop easily to these class of molecules. Hence, there has been tremendous research interest in this class of host–defense peptides.

The primary structures of a large number of endogenous peptides from insects to mammals have been determined[6–9,18]. Investigations of their secondary structures and membrane-binding and permeabilization properties have been the subject of research in an effort to understand the molecular mechanisms of action as well as target cell specificity[7,18–30]. 'Engineering' these peptides has also been another area of intense research with a view to generating molecules with improved antibacterial activity and also explore the possibility of using them as drugs to treat microbial infections. The approaches that have been employed in 'engineering' the biophysical properties which are responsible for biological activities that have emerged and the possible therapeutic uses of endogenous and 'designer' antibacterial peptides are discussed in this review.

Primary, secondary structures and mechanism of action of endogenous antibacterial peptides

Information about primary and secondary structures of endogenous antibacterial peptides and their mechanism of action have been important inputs in the engineering and design of analogues, variant and model peptides. Hence, these aspects are briefly reviewed in this section.

The primary structures of a large number of endogenous antibacterial peptides have been determined from insects, amphibians and mammals. An examination of the various sequences indicates that they can be classified into two groups, i.e. (i) linear peptides composed of ~ 20–50 residues, (ii) peptides with one to three disulphide bridges. Since details of the primary structures are available in recent reviews[6–9,18], only representative sequences are shown in Table 1. A striking feature in peptides belonging to both groups is the preponderance of cationic and hydrophobic residues, although there is no primary structure homology. Most of the antibacterial peptides have been isolated from the haemolymph of insects, skin, gastrointestinal tract of amphibians and neutrophils, macrophages or small intestinal paneth cells in mammals. A few peptides having antibacterial activity and rich in cationic amino acids have also been characterized from the seminal fluid of insects[31] and bovine species[32,33]. Peptide toxins like melittin (GIGAVLKVLTTGLPALISWIKRKRQQ–CONH$_2$) and pardaxin (GFFALIPKIISSPLFKTLLSAVGSALSSSGEQE) which are strongly haemolytic also possess potent antimicrobial activities[34–36]. In addition to antibacterial peptides composed of 20 or more residues, there are few with only ~ 13 residues like indolicidin[37] and bactenecin[38]. The antimicrobial spectrum and the potencies are highly variable. Cecropins and magainins act only on bacterial cells and not eukaryotic cells whereas defensins exhibit both antimicrobial and cytolytic activities. The antibacterial peptides exhibit a broad spectrum of activity against gram-negative and gram-positive microorganisms. Cecropins and magainins are more active against gram-negative compared to gram-positive microorganisms. Since the assay methods in the different studies vary, comparison of the minimal inhibitory concentration (MIC) in different studies may not be valid. The MICs of these class of peptides are in the µM range.

Although the primary structures of a large number of antibacterial peptides are documented, detailed secondary structures of only a few have been determined by nuclear magnetic resonance (NMR) or X-ray methods. Detailed below are structures of peptides that have been proposed based on NMR or X-ray methods. In the presence of structure-promoting solvents, cecropin A is proposed to adopt helical conformation with a hydrophilic N-terminal region (residues 1–21) connected by a hinge region (residues 22–24) to a C-terminal hydrophobic α-helical domain (residues 25–37)[39]. A continuous helical segment without the hinge has been proposed for cecropin P, a member of the cecropin family isolated from porcine small intestine[40]. Magainin 2 also adopts helical conformation in aqueous TFE mixtures[41]. However, recent studies in membranes employing Fourier transform infrared (FTIR) and solid-state NMR suggest two populations of the peptide, one in helical conformation and the other in β-sheet conformation[29]. Human defensin HNP-3 adopts β-sheet structure in the solid state[42] and in solution[22]. The structures of several defensins have been determined in solution by NMR (the results have been summarized in ref. 22). All α-defensins which have a conserved disulphide motif adopt β-sheet structure. β-defensins though possessing disulphide linkages different from α-defensins also adopt β-sheet structure. Insect defensins have regions of β-sheet and α-helical structure. NMR studies have indicated that a β-hairpin region composed of 18-residues is less flexible than the rest of the molecule, suggesting that this region may be an important determinant of activity. The toxins melittin and pardaxin also adopt α-helical conformation[43,44]. The central proline residue introduces a kink in both the helices. The structures of antibacterial peptides as deduced from NMR and X-ray studies are shown in

Table 1. Representative structures of endogenous antibacterial peptides

Peptide	Species	Sequence	MIC (µM)[c]	
			E. coli	*S. aureus*
Cecropin[i] A	*H. cecropia*	KWLFKKIEKVGQNIRDGIIKAGPAVAVVGQATQIAK-CONH$_2$	0.2	>200
Andropin[i]	*D. melanogaster*	VFIDILDKVENAIHNAAQVGIGFAKPFEKLINPK	140	11
Magainin-2[a]	*X. laevis*	GIGKFLHSAKKFGKAFVGEIMNS	1–7	na
Bombinin[a]	*B. variegata*	GIGALSAKGALKGLAKGLAEHFAN-CONH$_2$		
Dermaseptin[a]	*P. sauvagii*	ALWKTMLKKLGTMALHAGKAALGAADTISQGTQ	1.5	30
Seminalplasmin	Bovine	SDEKASPDKHHRFSLSRYAKLANRLANPKLLETFLSKWIGDRGNRSV	4	10
Indolicidin	Bovine	ILPWKWPWWPWRR-CONH$_2$	10	3
Defensin (α)	Human, HNP-1	ACYCRIPACIAGERRYGTCIYQGRLWAFCC	10	3
Defensin (β)	Bovine, BNBD-1	DFASCHTNGGICLPNRCPGHMIQIGICFRPRVKCCRSW	act	na
Defensin[i]	*P. terranovae*	ATCDLLSGTGINHSACAAHCLLRGNRGGYCNGKGVCVCRN	na	act
Tachyplesin[c]	*T. tridentatus*	KWCFRVCYRGICYRRCR-CONH$_2$	1.5	6
Protegrin-1	Porcine	RGGRLCYCRRRFCVCVGR	30	30
Brevinin 1-E	*R. esculenta*	FLPLLAGLAANFLPKIFCKITRKC	1	0.5

[i], insects; [a], amphibians; [c], The MIC values are not directly comparable as the strains against which different peptides were tested are not the same; act, active; na, not active; S–S bonds are indicated.

Figure 1. Circular dichroism (CD), infrared and Raman spectroscopy have also been employed to characterize the conformation of some of these peptides[7,18]. The acyclic peptides appear to favour helical conformation, especially in structure-promoting solvents and lipid vesicles.

The amphipathic nature of structures and the presence of cationic residues strongly suggest that interaction of endogenous antibacterial peptides with membranes mediates their biological activity. Both acyclic and cyclic peptides like the defensins bind to lipid vesicles and permeabilize them, resulting in the release of entrapped solutes[23–29,45]. Electrical conductance of bilayer membranes doped with these peptides shows characteristic features of single channel conductance[46–49]. Direct interaction and permeabilization of bacterial membranes has been demonstrated for peptides like defensins[6,50,51], tachyplesins[52], magainins[53,54] and seminalplasmin[55]. The recent observations that D-enantiomers of cecropins[56], magainins[57] and melittin[58] show antibacterial activity comparable to the L-enantiomers indicate that no chiral recognition is involved in their mechanism of action.

While it is evident that the mechanism of action of endogenous antibacterial peptides involves membrane permeabilization, the molecular architecture of the channels or pores formed in membranes is still debated upon. Magainins are presumed to bind to the membrane surface, which results in deformation and bending in a toroidal fashion, resulting in the formation of pores lined with polar head groups of the lipids[24,26] rather than trans-membrane channels formed by peptide aggregates like in alamethicin[12]. Cecropin P is presumed to bind to the membrane surface and destabilizes the phospholipid packing, leading to permeabilization. However, defensins are presumed to form transmembrane channels with the conducting unit being a dimer[42].

Antibacterial activities of peptides that correspond to segments of endogenous antibacterial peptides

Table 1 indicates that several acyclic peptides are composed of more than 25 residues which may not be necessary for interaction with membranes. Attempts to delineate short segments in these peptides that can bind

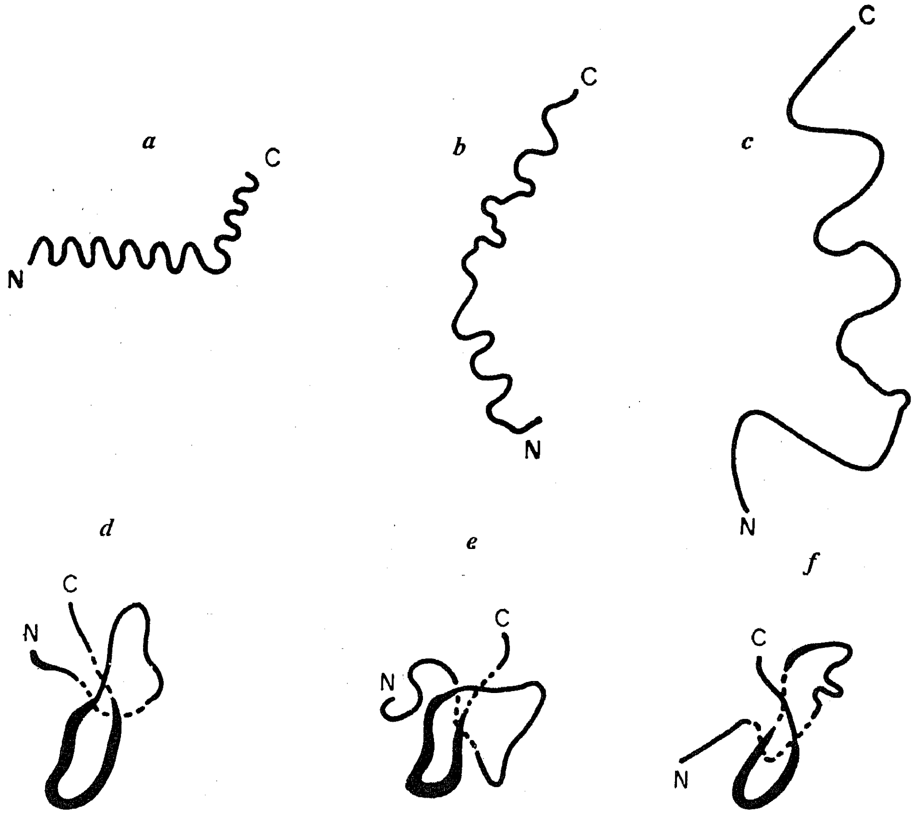

Figure 1. Schematic sketch of the secondary structures of peptides possessing antibacterial activity. *a*, cecropin A; *b*, melittin; *c*, pardaxin, *d*, α-defensin; *e*, β-defensin; *f*, insect defensin. Region indicated by thick line in defensin structures correspond to β-hairpin conformation which appears to be conserved. N and C indicate amino and carboxy terminii. The structures are not on the same scale.

to lipid vesicles and exhibit antibacterial activity have been successful. In SPLN (Table 1), hydrophobicity analysis has indicated a 27-residue stretch more hydrophobic than rest of the peptide. Synthetic peptides corresponding to the 27-residue segment and the 13-residue segment (PKLLETFLSKWIG) exhibited antimicrobial activity[59,60] though with less potency than SPLN. The short peptides bound to lipid vesicles and permeabilized bacterial membranes like SPLN[61]. Interestingly both peptides exhibited haemolytic activity not observed in the parent peptide.

Pardaxin is another molecule that has been investigated extensively[36,62,63]. The N-terminal 1–18 residues are essential for activity. Pardaxin and a peptide without the C-terminal seven amino acids exhibited potent antibacterial and haemolytic activities[35,36]. However, a peptide corresponding to the 1–18 segment showed selective antimicrobial activity with haemolytic activity even at high concentrations[64]. Peptides corresponding to the N-terminal segment composed of 14 or 15 residues, though could interact with model membranes did not possess antibacterial or haemolytic activities[65,66].

A synthetic peptide corresponding to N-terminal 18-residue segment of dermaseptin showed antimicrobial properties comparable to the parent 37-residue peptide[67]. Short peptides with deletions of amino acids from N or C terminal regions corresponding to cecropins[68–74] and magainins[3,75,76] have not yielded bioactive molecules. However, hybrid peptides composed of hydrophobic segments from cecropins and melittins[18,70,77,78] have been shown to possess antimicrobial activity. Thus, by judicious choice of hydrophobic or amphiphilic segments from antibacterial peptides, it is possible to generate endogenous bioactive peptides.

Engineering of 'short' bioactive peptides to improve potency and selectivity

Although peptides ~ 18 amino acids derived from longer peptides and some naturally occurring peptides like indolicidin ~ 13 residues long do have antibacterial activity, some of them also lyse erythrocytes and other eukaryotic cells. This property is not desirable if these peptides are to be used as drugs. Hence, there have been attempts to engineer such peptides to get desirable biological activities.

The 13-residue seminalplasmin-related peptide PKLLETFLSKWIG (SPF) had antibacterial and haemolytic activities at comparable concentrations[59]. A single amino change of glutamic acid to lysine resulted in considerable increase in antibacterial activity over haemolytic activity[79]. The activity profiles of several other analogues related to SPF[80] indicate that the aromatic residues were not important for antimicrobial activity. However, haemolytic activity was reduced considerably when the aromatic residues F and W were replaced by L. Likewise transposing KT and SK residues also favoured antibacterial over haemolytic activities. Replacement of K by E resulted in complete loss of biological activities.

Peptides having antibacterial and/or erythrocyte lytic activities could also be generated from the inactive N-terminal 16-residue segment of pardaxin, GFFALIPKIISSPLFK[81]. The analogue GFFKLIPKIIKSPLFK–CONH$_2$ showed selective antimicrobial activity whereas the analogues GFFKLIAKIIKSPLFK–CONH$_2$ and GFFAKIKKIIKSPLFK–CONH$_2$ exhibited both antimicrobial and erythrocyte lytic activities. In peptide amides corresponding to the 18-residue segment, GFFALIPKIISSPLFKTL[64], replacement of P (7) by A imparted haemolytic activity in addition to antibacterial activity. Antimicrobial activity but not haemolytic activity was abolished when K residues were replaced by Q.

Indolicidin is a peptide isolated from bovine neutrophils having an unusual amino acid composition[37]. Five of the 13 residues are W. There are also 3 P residues. The trp residues are essential for both the biological activities of indolicidin as replacement of W by F results in specific antibacterial activity[82]. The proline residues do not appear to be essential for activity as their replacement by A is tolerated. A similar study on the haemolytic peptide crabrolin (FLPLILRKIVTAL–CONH$_2$) has resulted in the identification of an analogue having specific antibacterial activity[83].

By making judicious change of amino acids, it is possible to convert an inactive peptide to an active one. For example, peptide corresponding to the ordered α-helical segment of the haemolytic toxin δ-haemolysin, IISTIGDLVKWIIDTV does not have erythrocyte lytic activity[84]. Analogues where D have been replaced with K showed potent antibacterial and haemolytic activities. Replacement of one or two D residues resulted in varying biological activities.

Several 15-residue peptides which are hybrids of melittin and cecropin have also been obtained possessing antimicrobial activities[70,78]. The segments from cecropin and melittin correspond to various hydrophobic regions in the parent peptides. There have been attempts to generate analogue with improved activity by replacement of amino acids in the full length cecropin, magainins[68–78] (summarized in ref. 18) and pardaxin[62]. Analogues of magainins have been obtained with considerably improved activity over the parent molecule[76]. However, in some analogues, improved antibacterial activity also resulted in increased haemolytic activities. In pardaxin, introduction of D-amino acids resulted in selective antibacterial activity over haemolytic activity[85]. Selective replacement of amino acids by D-isomers in melittin also resulted in selective lysis of bacteria but not mammalian cells[86].

Apart from efforts to engineer endogenous peptides by a rational approach to get short peptides with improved selective activity, there have been attempts to generate bioactive peptides by combinatorial methods[87–89]. Hexapeptides with good antibacterial activity, especially against *S. aureus* have been reported. However, the combinatorial method is restrictive and cannot be easily used for the synthesis of peptides composed of even 10 residues due to the large number of combinations involved.

Biophysical principles that govern the biological activities of antibacterial and haemolytic peptides

The membrane activity of several 'engineered' peptides described in the previous section has been investigated in detail[35,61,64–67,80–86,90]. The peptides bound to lipid vesicles and permeabilized them so as to cause the release of entrapped hydrophilic molecules like calcein or carboxyfluorescein. Interestingly, peptides that possessed both antibacterial and haemolytic activities were more effective in permeabilizing model membranes compared to peptides possessing only antibacterial activity. Now erythrocytes and other eukaryotic cells have efficient protein 'pumps' in the plasma membrane as a result of which small osmotic imbalance can be tolerated[91]. Hence effective permeabilization of the plasma membrane is required for lysis in the case of erythrocytes. However, the bacterial plasma membrane is the site of respiration[92] and even a small perturbation of the membrane could affect respiration. Thus, a peptide which is not an effective membrane perturbant could have selective antimicrobial activity. In many peptides the biological activities correlate with model membrane permeabilization. However, the molecular structure of the lesions formed by these peptides in membranes is yet to be established unequivocally.

The secondary structures of the various analogues and variants have been studied almost exclusively by CD spectroscopy[56–86]. The spectra suggest predominantly helical conformation for the peptides in trifluoroethanol and membrane environment. In water, almost all peptides show spectra which are characteristic of unfolded conformation. Helical content has been estimated from θ_{222} values. Since there are inherent limitations in quantitating secondary structure from CD data, the molecular structures of the peptides cannot be commented upon. However, as large number of related peptides with varying activities have been studied, their results do permit some correlation of structures with activity. A striking observation is that several peptides that have only antibacterial activity have less propensity for helical conformation than peptides which are strongly haemolytic in addition to possessing antibacterial activity. In fact, several analogues of magainins[76] that have enhanced antibacterial activity are also haemolytic and are more helical than the parent peptide. In melittin[86] and pardaxin[35,85], introduction of D-amino acids, which result in decrease in helical content, show selective antimicrobial activity. However, there are exceptions to the above mentioned observations. For example, dermaseptin and its N-terminal 18-residue peptide, though exhibit only antibacterial activity show high helical content in trifluoroethanol[67]. However, it is evident that the acyclic peptides adopt predominantly helical structure in hydrophobic environment and are largely unordered in aqueous medium. Lower helical content also correlates with lower membrane-perturbing ability.

In summary, lower helical content and consequently less membrane-perturbing ability favours specific antibacterial activity. For haemolytic activity, high helical content appears to be necessary. The presence of cationic residues is essential for antibacterial activity but not for haemolytic activity. Studies on peptides containing disulphide bridges have largely been confined to the naturally isolated ones. These peptides adopt β-sheet structures and breaking of the disulphide bridges leads to unfolding and loss of biological activities[6,8,9].

Do endogenous antibacterial peptides or their 'engineered' variants have potential as therapeutic agents?

The 'endogenous' host–defense peptides and the 'engineered' synthetic peptides have broad spectrum antibacterial activities against a variety of gram-negative, gram-positive microorganisms and fungi. Anti-viral activity has also been observed in some peptides. Clinically isolated strains that are resistant to conventionally used drugs are also susceptible to these peptides. Magainins and their analogues have been shown to have antitumour activities against murine and human cell lines[93–96]. *In vivo* activity has been demonstrated in a murine model[96]. By suitable engineering based on biophysical principles, it is possible to eliminate cytolytic activity against eukaryotic cells and also reduce the length of the peptides substantially compared to endogenous peptides. The problem of inactivation of the all L-peptides by proteases can also be overcome by selective incorporation of D-amino acids or non-coded amino acids. As they are part of the endogenous host–defense mechanism, toxicity is unlikely to be a serious problem. In the case of indolicidin which has cytotoxic activity in addition to its antibacterial activity, entrapment in liposomes reduced cytotoxicity considerably and was effective in treating animals systemically infected with *Aspergillus fumigatus*[97]. By appropriate formulation, it should be thus possible to reduce toxicity when present and render the peptides suitable for systemic use. Hence, these class of antibacterial agents have all the features requisite for use as therapeutic agents. To-date, no reports of hormone action or undesirable immune reaction related to these class of peptides have been reported. Yet, to-date either endogenous or engineered antibacterial peptides have not found extensive use as antibacterial drugs for systemic or even topical applications. The one factor that could come in the way of their use as drugs is the costs involved in bringing an antibiotic drug to market[98]. It appears that costs have to be less than US $ 10 per gram to be competitive with other drugs in clinical use[8]. However, the dictates of economy should not come in the way of using peptides as drugs considering the serious problem of resistance to currently used antibiotics, especially when the immune system is compromised like in AIDS. Defensins have also been shown to act against non-tuberculosis mycobacteria[6] and hence could be active even against *M. tuberculosis*. Since resistance to several currently used drugs by *M. tuberculosis* is also being recognized as a serious health problem[99], there is an urgent need to try novel approaches to combat bacterial infections. Even the fluoroquinolone class of antibiotics are not refractory to the development of resistance. Hence, antibacterial peptides that act by permeabilizing membranes are promising alternatives to currently used drugs. Economics should not overrule an obvious scientific advantage.

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Structural studies of protein unfolding

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Methods for macromolecular structure determination (NMR and crystallography) are now being used to get structural information on partially folded and unfolded states of proteins. These techniques, in combination with proton hydrogen exchange studies are powerful tools to extract information on non-native states of proteins. This review discusses progress in this area of protein folding.

PROTEINS are biopolymers of twenty naturally occurring amino acids. Protein chains are typically between 50 and 500 amino acids in length. Each protein is characterized by one (or in a few cases, more than one) folded chain conformation. An important unsolved biological problem, called the 'protein folding problem'[1–3], is understanding the relationship between the amino acid sequence of a protein and its folded three-dimensional structure. At present (and in the foreseeable future) the number of known protein sequences is far in excess of the number of known protein structures. Protein sequences can be readily determined from the DNA sequence of the corresponding gene. In contrast, protein structures can be determined to atomic resolution only by X-ray crystallography or nuclear magnetic resonance (NMR) techniques. Both these techniques are time consuming, technically difficult and require large amounts (10–100 mg) of protein. Since protein structure is intimately linked with biological function it would be highly desirable to predict structure from the amino acid sequence.

Protein folding is a search for the final folded state starting from an unfolded, ensemble of conformationally flexible states. A folded protein chain can theoretically adopt a very large number of sterically allowed conformations. However, many proteins are able to fold from an unfolded random coil state to the final folded state on timescales ranging from milliseconds to minutes. In these timescales only a tiny fraction of all sterically allowed conformations can be sampled. Protein folding is often well described by a two-state process in which only the folded state N (with well-defined three-dimensional structure) and the unfolded state U (which consists of an ensemble of interconverting conformations) are significantly populated. An understanding of the pathway(s) by which a given protein folds might be useful in the solution of the protein folding problem[2,3]. However, because the protein folding process is rapid and often takes place without detectable intermediates (states different in structure or energetics from both the folded and the unfolded state), this is a challenging task. Much effort has gone into identifying solution conditions (pH, temperature, denaturant or additive concentration) under which folding intermediates are populated to a significant extent, either kinetically or at equilibrium[4,5]. If conditions can be identified under which folding intermediates are in equilibrium with either the folded or the unfolded state, then the conformation of such states can be probed by a variety of spectroscopic and hydrodynamic techniques. In such cases it is important to show that such equilibrium intermediates are similar to intermediates formed along the kinetic pathway of folding of the protein[6]. It is also of interest to characterize the thermodynamic parameters (especially the free energy changes) involved in transitions from unfolded and partially folded states to the folded state. As summarized below, denaturants such as urea, guanidine hydrochloride (GdnCl), pH or temperature are used both in kinetic and thermodynamic studies of protein folding[2,7].

In order to measure the free energy of folding of a protein (ΔG^0), it is necessary to find a range of conditions under which both the folded state (N) and the unfolded state (U) are populated to significant extents. The equilibrium constant $K_f = [N]/[U]$ can then be accurately determined as a function of denaturant concentration or temperature. However, the values of ΔG^0 ($\Delta G^0 = -RT \ln K_f$) measured under these conditions need to be extrapolated to zero denaturant concentration, neutral pH and room temperature. In the case of urea and guanidine hydrochloride denaturation, a linear extrapolation procedure is widely used, $\Delta G^0_D = \Delta G^0 + m^*[D]$, where ΔG^0_D is the free energy of folding at a denaturant concentration [D] and m is the slope of a plot of ΔG^0_D vs [D]. Despite their extensive use, the structural basis of denaturation by urea and GdnCl is still not clear[8,9]. Structural information has been obtained indirectly by biophysical studies like spectroscopy[10] and thermodynamics[11] .There are two mechanisms by which urea is thought to destabilize proteins. Urea may interact with backbone peptide groups through the formation of hydrogen bonds. The crystal structure of the diketopiperazine–urea complex[12] shows that the diketopiperazine molecule forms several hydrogen bonds with urea molecules. Solvation of the diketopiperazine by urea is so extensive that there are no hydrogen bonds between diketopiperazine molecules in the crystal. However it should be noted that this structural information is derived from a tightly packed small molecule crystal comprising solely of urea and diketopiperazine. The interactions of urea with the peptide backbone in aqueous solution where a large amount of aqueous hydrogen bonding solvent is present, may be quite different. Another proposed mechanism for urea denaturation is that high concentrations of urea decrease the magnitude of the hydrophobic driving force. Support for this comes from solubility data of hydrocarbons in urea[13]. Thermodynamic studies of the binding of urea to proteins and peptides[11] suggest that urea binds weakly to the protein with dissociation constants in the molar range. A study of the specificity of urea binding to the folded states of two small proteins, BPTI and PEC-60, was recently carried out by monitoring the NMR chemical shifts[14] of protein protons as a function of urea concentration. Binding constants obtained by this technique were similar to those obtained calorimetrically[11]. However, neither of these two proteins is denatured by urea. In order to clarify the mechanism of urea denaturation, it is important to carry out structural studies of proteins and peptides that undergo denaturation in the presence of urea. Although NMR and X-ray crystallography are the two most popular tools for macromolecular structure determination, there are problems with application of these techniques to determining the structure of unfolded and partially folded proteins. Such states generally do not show well resolved NMR peaks and display much smaller dispersion in chemical shifts than in the folded state. No partially unfolded state of a protein has been crystallized to date, probably because disordered regions of the protein act as an impediment to crystallization. Partially unfolded states also show a tendency to aggregate at the high concentrations required for crystallography and NMR. However, with increases in the magnetic field strengths available as well as with the advent of heteronuclear NMR experiments it has been possible to assign spectra of proteins in the unfolded state where there is extensive resonance overlap due to conformational averaging[15–17]. This is the first step in structural characterization of the unfolded state though much progress remains to be made. NMR has also been used to provide indirect structural information on unfolded and partially folded states through hydrogen exchange studies.

One of the most informative techniques used in studying the process of protein folding is that of hydrogen exchange[18–22]. Protons in a protein that are bonded to nitrogen or oxygen atoms can exchange with protons from the aqueous solvent. The rate at which exchange occurs is determined by several factors such as solution pH and temperature, the accessibility of a given proton to solvent, whether the proton is hydrogen bonded to another acceptor in the protein and the conformational stability of the protein. For many protons on the surface of the protein, exchange is a rapid process that occurs in less than a second. Interior protons have much slower exchange rates and in some cases half lives of exchange can be several months. A commonly used model for describing hydrogen exchange is as follows. The hydrogen exchange rate constant of an amide proton that is fully accessible to solvent is given by,

$$k_3 = k_a\,[\mathrm{H}^+] + k_b\,[\mathrm{OH}^-] + k_w \qquad (1)$$

For a protected amide proton exchange can be modelled as shown below,

$$\mathrm{C} \overset{k_1}{\underset{k_2}{\Leftrightarrow}} \mathrm{O} \overset{k_3}{\Rightarrow} \mathrm{EX} \qquad (2)$$

Here C and O are the closed and open states for a given amide proton. Exchange is assumed to take place only from the open state with a rate constant of k_3. There are two limiting cases for this model. In the first case, the so-called EX1 limit, $k_2 \ll k_3$. Then the overall rate constant for exchange k_{ex} is equal to k_1, the rate constant for opening of the protein to an exchange competent state. In the second limiting case, the so-called EX2 limit, $k_3 \ll k_2$. Hence $k_{ex} = (k_1/k_2)*k_3 = K*k_3$, where K is the equilibrium constant for the transition between C and O. Exchange rate constants for exchange that occurs by the EX1 mechanism will be relatively independent of pH. In contrast, for the EX2 case, the exchange rate constant is expected to depend on pH according to equation (1). In the majority of cases, exchange appears to take place by the EX2 mechanism though cases of EX1 exchange have also been observed[22,23]. Values of k_3 for a given amide proton can be estimated from exchange rate measurements in small peptides[24]. Thus measurements of k_{ex} for EX2 exchange can be used to determine the equilibrium constant K for unfolding to an exchange competent state. Much recent work has focused on measurements of exchange rates of the native state (and hence K and ΔG^0 and m for the C to O transition) as a function of denaturant concentration[23,25–27]. These exchange rates for individual amide protons can be made using 2D NMR. Proteins studied include RNase H (ref. 28), RNase A (ref. 29) and Cyt c (ref. 30). From such measurements it is possible to determine whether an exchange event for a given proton is coupled to global or local unfolding. ΔG^0 and m values for individual amide protons determined from such exchange measurements can be compared with the ΔG^0 and m values obtained from spectroscopic measurements of global protein folding. If the two values are identical, this suggests that the O state for the amide proton is identical to the globally unfolded state. This method of obtaining information about folding intermediates and local unfolding is termed 'native state' exchange because studies are carried out at low denaturant concentrations in which most protein molecules are in the native state.

Another hydrogen exchange technique that has provided much useful information is the use of NMR to determine at what stage during the folding pathway a specific amide proton becomes protected from exchange[29–32]. As a protein folds, some amide protons become inaccessible to further exchange. By appropriately changing the pH during the course of folding, it is possible to restrict exchange to occur only at defined periods during the folding process. The NMR spectrum can be acquired once the molecule has folded. The extent of exchange occurring during the exchange period can therefore be quantified. From this information it is possible to obtain a qualitative idea of the structure present in the protein during the time interval that exchange occurred[31,32]. In cases where equilibrium intermediates have been identified, hydrogen exchange studies[6,20,33] have also been used to provide indirect structural information about such molecules.

X-ray crystallography is widely used to determine macromolecular structure. However it has not been widely used to study the protein folding process or to examine the effects of denaturants on protein structure. This technique provides information complementary to that obtained from NMR. Unlike NMR, which is currently limited to proteins of molecular weight less than 30 kD, crystallography has no size restriction. While NMR hydrogen exchange studies monitor primarily the dynamic behaviour of amide protons, in crystallography it is, in principle, possible to obtain the structure of the protein at atomic resolution as a function of denaturant concentration. While NMR can also be used for protein structure determination, the positional accuracy of coordinates obtained by this technique is still not comparable to that of a high resolution crystal structure.

Crystallographic studies in the presence of denaturants are limited to the study of a peptide model in urea[12], high resolution crystal structures of hen egg white lysozyme in the presence of denaturant concentrations as high as 8 M (ref. 34) and a low resolution structure of alpha chymotrypsin in the presence of GuHCl and urea[35]. In the case of lysozyme little change in protein structure was observed, probably because lysozyme is stable to urea denaturation at room temperature and physiological pH. With an increase in urea concentration, an increase in the order of the protein chain was observed in the loops and a few ordered urea molecules could be seen in the structure. Many of these occurred at bridging positions between symmetry-related molecules in the crystal. In the case of α-chymotrypsin, the protein undergoes denaturation in the presence of urea and it was not possible to collect high resolution X-ray data. This study was performed several years ago. Since then, data collection and refinement techniques have improved considerably. It is now possible to routinely collect datasets in a day, instead of in several weeks as was the case previously.

We have recently determined the structure of the protein–peptide complex RNase S in different concentrations of urea (Ratnaparkhi and Varadarajan, unpublished results). The objective of these studies is to obtain information on the structural changes associated with urea denaturation of this molecule. RNase S consists of S peptide (residues 1–20) and S protein (residues 21–124), two proteolytic fragments of the 124 amino acid protein, RNase A (Figure 1). These fragments are obtained by cleavage of the peptide bond between Ala-20 and Ser-21 of RNase A by subtilisin. The two fragments can be reconstituted to give a non-covalently bound, active, S peptide, S protein complex, RNase S (ref. 36). The residues 16–20 of the S peptide are not involved in binding of S peptide to S protein. The refined

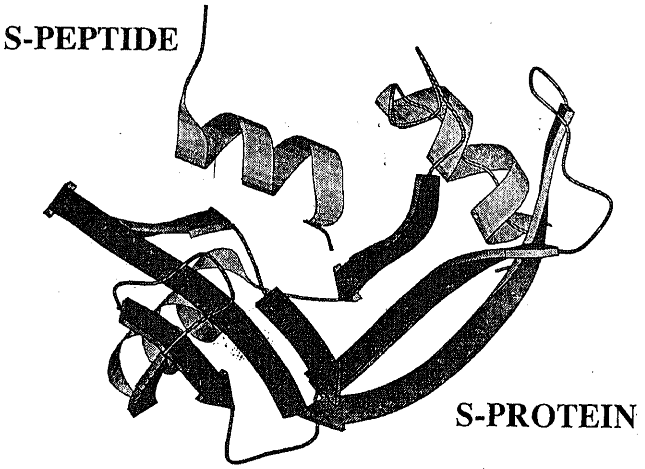

Figure 1. Ribbon diagram of ribonuclease S (ref. 38) drawn using the program MOLSCRIPT.

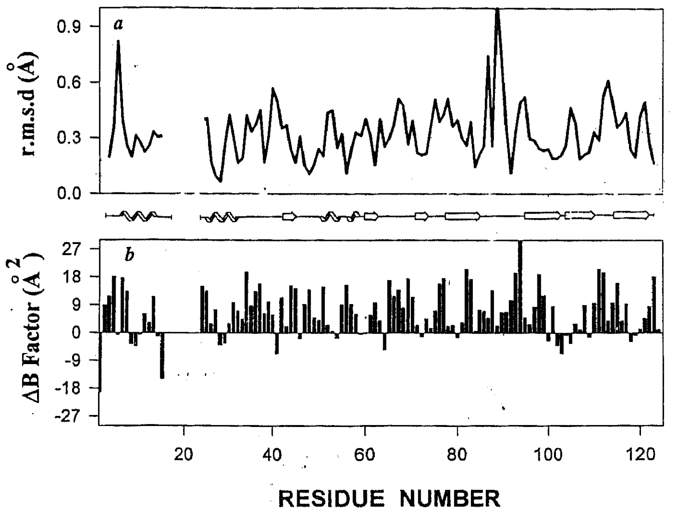

Figure 2. Plots representing the structure of RNase S in 5 M urea. The secondary structure along the sequence is represented as follows; Helix { ∿ }, beta-sheet {⇨}, loops {—}. The break in the graph is because of lack of electron density for residues 16–23. ***a***, Root mean square derivation plot; ***b***, ΔB factor plot.

three-dimensional structures of RNase A and RNase S are now available to a high resolution[37,38] and the two proteins have very similar structures. We have previously characterized the thermodynamic and structural changes associated with a large number of residue substitutions in RNase S (refs 39–42). Thermodynamics of binding of various S-peptide analogues to S-protein were measured by titration calorimetry and structures of the bound complexes were characterized by X-ray crystallography. A significant advantage of the RNase S system is that it is a bimolecular system and hence effects of mutations on molecular stability can be studied by isothermal titration calorimetry[39]. In contrast, for most proteins, protein folding is a unimolecular reaction. Hence mutational effects on stability are typically studied using either spectroscopic or differential scanning calorimetric measurements. Measurements of important parameters such as the ΔH^0, ΔG^0 and ΔC_p of folding made by titration calorimetry are considerably more accurate than measurements made using either differential scanning calorimetry or spectroscopy. RNase S is readily crystallized and crystals typically diffract to a resolution of better than 1.8 Å. In solution at pH 8, RNase S is 50% denatured at a urea concentration of 3.5 M. We have soaked crystals of RNase S in a range of urea concentrations (0 M, 1.5 M, 2 M, 3 M and 5 M) and have solved these structures to resolutions ranging from 1.8 to 3.0 Å. At urea concentrations greater than 2 M, crystals were stabilized by crosslinking with glutaraldehyde before soaking in urea. In each case, a control data set was collected on a crystal crosslinked for an identical period of time but which was not soaked in urea. Crosslinking for up to 30 min had no effect on either the resolution of the data or the three-dimensional structure of the molecule. At higher crosslinking times (greater than 40 min) there was a rapid decrease in the resolution to which the crystals diffracted. In the urea-soaked crystals, the largest changes were observed in the structure of the complex at 5 M urea. Shown in Figure 2 *a* is the root mean square difference in coordinates of the main chain coordinates of the 5 M urea structure relative to the control structure in the absence of urea. These differences are plotted as a function of residue number. Figure 2 *b* shows the difference in the average main chain B factor of the 5 M urea structure relative to its control. Also indicated at the top of the figure are the positions of α helices, β strands and loops/turns in the sequence. There are significant changes in both the main chain coordinates and in the B-factors relative to the control structure. These differences are largest for the loop regions, especially for the loop between residues 65 and 72. Interestingly, there are also large changes observed in the position of this loop in mutants of RNase S which are significantly destabilized with respect to the wild type complex[41], suggesting that movement of this loop may be an initial step in the unfolding of the complex. It is also of interest to note that the β strand regions on average show smaller increases in B-factor than the α helical regions. These results are consistent with earlier exchange studies of RNase A (ref. 31) which show that the β sheet appears to be protected at earlier times in folding than any of the α helices. These results need to be substantiated by solving other RNase S crystal structures as a function of denaturant concentrations in different crystal forms. X-ray data for the structures discussed above were collected at room temperature using a rotating anode X-ray source. Recent advances in X-ray crystallography include the use of more powerful X-ray sources, flash freezing techniques to reduce radiation decay as well as techniques such as multiwavelength anomalous dispersion (MAD) phasing to obtain a more accurate image of the structure and solvent without calculation of phases[43] from a model. Our work demonstrates that it is possible to use crystallography to solve protein crystal structures in the presence of denaturants and we anticipate that such structures will provide a wealth of information about the structural basis for denaturation by different denaturants as well as on partially folded states of proteins.

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Molten globule state of human serum albumin in urea

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Preferential interaction and hydration parameters of human serum albumin (HSA) as a function of urea concentration in the entire solubility range are obtained by partial specific volume measurements. At lower concentrations (< 2 M) of urea, HSA exists in a molten globule state with an unusually hydrated structure and increased Stokes radius. Differential mechanism of interaction of urea at low and high concentrations with HSA exists. This is in tune and conformity with the first observation made by Prakash and Timasheff for other proteins.

THE role of classical protein denaturants such as urea and guanidine hydrochloride has always been one transition of the native to denatured state. But in this large concentration of the denaturants, there are many intermediates that exist, which are identified and characterized[1]. One of the main difficulties in experimental investigations of folding and unfolding of proteins is the reliable identification and structural description of the intermediate state/s. But what interests the authors is – at very low concentration of denaturants what kind of intermediates are formed? How stable are they? What is the relevance of such intermediates to molten globule state? Hence it was thought to investigate especially in urea the fate of human serum albumin (HSA) at low concentration of this denaturant as the availability of the data has been scantier and few references have shown that such intermediates are possibly existing.

Urea is a denaturant which can bind to proteins, reversibly in many cases and is exploited to understand the mechanism of protein folding. Although considerable progress has been made in experimental studies of the folding and unfolding, our knowledge is limited by the difficulty in obtaining structural and kinetic data of folding intermediates in solution or through crystallographic studies. Hence more detailed studies are needed to understand the role of solvent and solute with proteins in solution. In this direction, numerous studies have dealt with the behaviour of different proteins in urea solution, but the total mechanism of interaction of urea with proteins is poorly understood.

To understand the forces governing the formation of three-dimensional architecture of proteins from the primary structure, studies were focused on the characterization of denatured structure and unfolding/refolding intermediates under several non-native conditions such as high ionic strength, extremes of pH, organic solvents, temperature/pressure variations and mild denaturant conditions[2]. In this direction, efforts were made to look for the solution behaviour of HSA in urea.

Stabilization of proteins at lower concentration of urea has been of major concern today, with many reports showing the urea-induced stability of a variety of proteins with many different experimental approaches[3,4]. These intermediates in the protein unfolding pathway include *molten globule* state which is characterized at low concentration of urea[5–9]. But none of these reports precisely establish the mechanism of interaction of urea at low concentration with proteins including its relevance in forming *molten globule* structures. There were few evidences where many multimeric seed proteins aggregate at low concentration of urea exhibiting the *molten globule* properties. In this context it was important to obtain quantitative preferential interaction data and structural properties of HSA in the entire solubility range of urea.

However, the interaction of urea at low concentrations[10] has been shown for the first time by Prakash and Timasheff[10] that there are two kinds of interactions, namely initially one that is dominated by preferential hydration at low concentration later, normal preferential interaction overrides[10]. This has been shown in case of RNase and lysozyme[10].

Preferential interaction of urea and volume changes in proteins are the subject matter of study in a number of proteins establishing mechanism of denaturation[11,12]. However, the interaction of urea with HSA at lower concentrations is not clear. Free energy of stabilization of proteins in denaturants is commonly calculated using the two state model $N \rightleftharpoons U$ and the role of Wyman linkage in such a path and also assuming the linear dependency of ΔG_{stab} with denaturant concentrations[13–17]. However, it is observed that the linear extrapolation of ΔG_{stab} to lower concentrations (< 2 M) of denaturants was not fitting into linear equations as it fits in at higher concentrations[18]. There are few evidences[19] to show that the mechanism of interaction of urea at lower concentration seems to be different compared to higher concentration. It was also observed as hydrophobic interaction in the case of multisubunit proteins, where it aggregates at a narrow range of lower concentration of urea[20]. In the case of HSA, the effect of low concentration of urea has been studied to understand the phenomena of intermediate/s, especially from the point of view of hydration and volume changes. Urea–HSA interaction system is studied at lower concentration of urea, using preferential interaction parameters and chromatographic techniques. The data at higher concentration of urea is also derived to explain the structural state of HSA in these concentrations of urea.

Methodology

Materials

Human serum albumin, Cohn fraction V (essentially fatty acid free, lot 42 H9313) and urea (83 F0483) were obtained from Sigma, USA. HSA was passed through Sephadex G-100 and the separated monomer was dialysed against triple glass distilled water at 4°C for 24 h and checked for homogeneity. The urea solutions were prepared according to standard procedures[21,22]. Dialysis membrane was obtained from Spectrum, USA. The buffer salts were of analytical grade obtained from Merck India Ltd, Mumbai, India. Triple glass distilled and deionised water was used in all the experiments.

Preferential interaction of urea

Preferential interaction parameters were obtained from densimetry method using Anton Paar DMA-58 ultra-precision densitymeter at 20 ± 0.01°C according to standard procedures[23,24]. The data was analysed for thermodynamical parameters using a three-component system: water, protein and denaturant as components 1, 2 and 3 respectively, following the standard notations[25,26].

Size-exclusion HPLC studies

HPLC experiments were carried out using a Shimadzu LC-RA series chromatographic system with a SPD-6AV ultraviolet detector. A Progel-TSK, G2000 SWXL (300 × 7.8 mm) (Supelco) column was pre-equilibrated with buffer containing specific concentrations of urea before loading the protein. HSA dissolved in buffer containing specific concentrations of urea and dialysed against the same for 24 h at 10°C. The dialysed samples were centrifuged at 10,000 rpm for 1 h at 10°C before loading on to the column and the exact protein concentration in urea was determined with appropriate corrections for molar absorption coefficient of HSA. In all the experiments, 50 μl of HSA of concentration 20 mg/ml and a flow rate of 1.5 ml/min was used. The Stokes radius of HSA was calculated using standard procedure[27].

In order to quantitate the urea interaction and see the hydrodynamic changes in the HSA molecule, partial specific volume measurements were made both at constant molal and constant chemical potential conditions. The density of different HSA concentrations ranging from 5 to 25 mg/ml in single urea concentration was measured and converted to partial specific volume using the standard method described under materials and methods. The isomolal value remained fairly constant in all the urea concentrations below 8 M. But the isopotential values changed significantly. The values of HSA for isomolal and isopotential conditions, respectively, for different concentrations given are 1 M (0.735 ml/g, 0.739 ml/g), 6 M (0.733 ml/g, 0.722 ml/g) and 8 M (0.734 ml/g, 0.730 ml/g) respectively. These are the average values of four identical experiments with an error bar of ± 0.001 ml/g. It is well documented in the literature that normally increased partial specific volume of protein under isopotential conditions in a three-component system indicates the preferential hydration of the macromolecule[28].

Table 1 shows the changes in preferential interaction parameters (ξ_3) with increasing urea concentration having more data points below 3 M. There was a negative preferential interaction of HSA till 2 M concentration of urea where it reaches a maximum with ξ_3 value of – 0.0215 ± 0.0063 g/g. Above 2 M concentration, preferential interaction of urea predominates as observed by the positive values of ξ_3. The isomolal value of HSA remained fairly constant but isopotential values increased below 2 M concentrations of urea. There is a steep increase in the preferential interaction of urea between 2 M and 6 M, reaching a maximum at 6 M with the ξ_3 values of 0.0646 g/g. Preferential interaction parameter and related parameters with error bars at low and high concentrations of urea at 20°C are shown in Tables 2 and 3 respectively.

At low concentration of urea up to 2 M, there is decrease in ΔV values, reaching a maximum value of – 233 ± 16 ml/mol and the transition passes through to the positive side at 3 M urea with a value of 199 ± 18 ml/mol. This transition is possibly due to changes in the transition from hydrated molecule at lower concentration of urea to that of the unfolded molecule at higher concentration of urea and an equilibrium existing between the two. And the equilibrium shifts toward the unfolding process as the concentration of urea increases. The ΔV reaches a maximum value at around 5.6–6 M, after which it decreases with a value at 6 M being 997 ± 60 ml/mol. This decrease in the ΔV value after reaching a maximum is possibly due to the unfolding of protein molecule and exposure of a number of both hydrophobic and hydrophilic sites to bulk solvent, leading to the net change in the preferential interaction, which is lower. At higher concentration, the data follows a direct relation between ΔV and urea concentration as observed for other proteins also[11].

The size of the protein was determined by a direct method of size exclusion HPLC and the Stokes radius of HSA was calculated. Figure 1 shows the Stokes radius of HSA at different concentrations of urea. HSA at lower concentrations of urea eluted much earlier with lower distribution coefficients than the HSA in native buffer. At 0.5 and 1.6 M of urea there was 9 and 6% increase, respectively, in the Stokes radius of HSA molecule compared to native HSA, whereas at 4 M about 15% increase was seen. Urea at these concentrations did not affect the permeation capabilities of the stationary phase of the column.

Table 1. Preferential interaction parameter of human serum albumin in aqueous urea at selected lower and higher concentrations

Molarity of urea	0.5	0.8	1.0	1.6	2.0	3.0	4.5	6.0	7.0	8.0	9.0	10.0
$(\delta g_3/(\delta g_2)T$, μ_1, μ_3 (g/g)	0.009 ± 0.002	0.012 ± 0.008	0.015 ± 0.008	0.017 ± 0.004	0.022 ± 0.006	−0.009 ± 0.003	−0.026 ± 0.007	−0.065 ± 0.014	−0.056 ± 0.013	−0.028 ± 0.009	−0.012 ± 0.004	0.046 ± 0.028

Table 2. Partial specific volume, preferential interaction parameter and related parameters of HSA interaction at specific lower concentration of urea

	Molarity of urea					
Interaction parameter	0.5	0.8	1.0	1.6	2.0	3.0
ϕ_2^0 (ml/g)	0.735 ± 0.001	0.735 ± 0.001	0.735 ± 0.002	0.734 ± 0.001	0.735 ± 0.001	0.734 ± 0.001
$\phi_2'^0$ (ml/g)	0.735 ± 0.001	0.738 ± 0.001	0.739 ± 0.001	0.738 ± 0.001	0.740 ± 0.001	0.732 ± 0.001
g_3 (g/g)	0.03 ± 0.01	0.05 ± 0.01	0.06 ± 0.02	0.10 ± 0.03	0.13 ± 0.03	0.21 ± 0.04
m_3 (mol of solvent per 1000 g H_2O)	0.52 ± 0.08	0.83 ± 0.16	1.06 ± 0.28	1.72 ± 0.32	2.19 ± 0.40	3.43 ± 0.60
$(\delta g_1/\delta g_2)T$, μ_1, μ_3 (g/g)	0.274 ± 0.033	0.242 ± 0.036	0.241 ± 0.068	0.161 ± 0.049	0.163 ± 0.060	0.045 ± 0.17
$(\delta m_3/\delta m_2)T$, μ_1, μ_3 (mol/mol)	9.37 ± 2.02	13.28 ± 3.02	17.04 ± 4.32	18.34 ± 5.02	23.73 ± 5.95	−10.33 ± 2.90
ΔV (ml/mol)	−126 ± 10	−199 ± 13	−233 ± 16	−199 ± 13	−332 ± 19	+199 ± 18

Table 3. Partial specific volume, preferential interaction parameter and related parameters of HSA interaction at specific higher concentration of urea

	Molarity of urea					
Interaction parameter	4.5	6.0	7.0	8.0	9.0	10.0
ϕ_2^0 (ml/g)	0.734 ± 0.001	0.733 ± 0.001	0.734 ± 0.001	0.734 ± 0.001	0.734 ± 0.001	0.730 ± 0.001
$\phi_2'^0$ (ml/g)	0.729 ± 0.001	0.722 ± 0.001	0.727 ± 0.001	0.730 ± 0.001	0.733 ± 0.001	0.736 ± 0.001
g_3 (g/g)	0.34 ± 0.06	0.49 ± 0.08	0.61 ± 0.10	0.75 ± 0.20	0.91 ± 0.22	1.09 ± 0.26
m_3 (mol of solvent per 1000 g H_2O)	5.62 ± 0.60	8.17 ± 1.20	10.15 ± 1.60	12.41 ± 1.80	15.16 ± 2.00	18.08 ± 2.60
$(\delta g_1/\delta g_2)T$, μ_1, μ_3 (g/g)	0.078 ± 0.032	0.132 ± 0.059	0.091 ± 0.040	0.041 ± 0.019	0.013 ± 0.020	0.042 ± 0.016
$(\delta m_3/\delta m_2)T$, μ_1, μ_3 (mol/mol)	−29.20 ± 4.10	−71.42 ± 6.57	−61.44 ± 7.10	−30.44 ± 8.50	−13.10 ± 8.00	50.69 ± 9.05
ΔV (ml/mol)	+399 ± 20	+997 ± 60	+598 ± 35	+365 ± 25	+166 ± 10	−102 ± 8

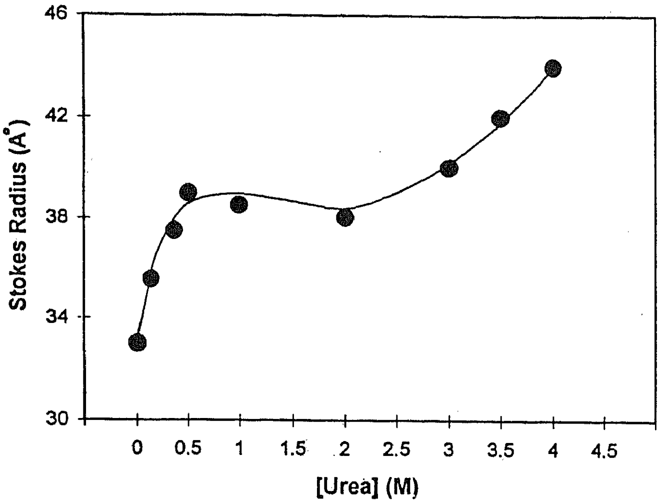

Figure 1. Stokes radius of human serum albumin as a function of urea concentration calculated from size exclusion–HPLC experiments. Urea did not alter the permeation properties of the gel matrix.

These results show a transition in the size of the protein which initially increases by about 6 Å units up to a concentration of 1 M urea, above which it plateaus and above 1.5 M urea there is a constant increase in the average size of the protein. It is significant to note that the initial increase in the size of the molecule is due to the hydration status of the molecule, possibly in the *molten globule* state. At higher urea concentrations, the HSA size increases in a manner similar to that observed for a large number of proteins.

Preferential exclusion overrides the preferential interaction of urea, resulting in the net decrease of preferential interaction above 8 M concentration of urea when compared to binding at the plateau region. Therefore, for any meaningful evaluation of preferential interaction or preferential hydration data, one has to evaluate the ξ_3 value over the entire concentration range of denaturant and not at single point measurement, indicating the nature of subtle changes that can occur in the protein as reflected in the ξ_3 results in the present study. Here there is a fine balance between binding and exclusion. These observations are similar to the interaction of $MgCl_2$ (ref. 28) and DMSO[29] with different proteins. The thermodynamics and the molecular meaning of binding are explained clearly in a three-component system[30].

The sedimentation velocity data was collected for HSA both at native state and at 1.8 M urea concentration where maximum changes were observed in terms of preferential interaction/preferential hydration and Stokes radius. The sedimentation coefficient decreased from a native $S_{20,w}$ value of 4.2 S to 3.8 S. This can only be possible if the molecule has an increased buoyant density, $(1 - \bar{V}\rho)$, of the solution. The increased hydration shell of the protein must be the driving force to increase the buoyant factor, thus decreasing the sedimentation coefficient of the protein molecule.

The results suggest that, as indicated in the case of RNase and lysozyme[10] and in HSA also there are two major structural features in urea. One, *molten globule* state of the protein at lower concentration of urea and second, the unfolding process of the protein leading to the denatured state at higher concentration of urea. There is a remarkable transition between the two structures as evidenced by the above data, which clearly indicates the role played by water at lower concentration of urea in increasing the hydrodynamic volume of the protein and secondary structural alterations typical to *molten globule* state. At higher concentration of urea the profile indicates the typical denaturation process as seen in large number of proteins. These data have substantial thermodynamic implications in the behaviour of proteins at lower concentration of non-electrolyte denaturant like urea and can only be explained by the existence of *molten globule* state of HSA in these concentrations of denaturant.

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Intracellular dynamics seen through time-resolved fluorescence microscopy

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The application of time-resolved fluorescence microscopy in cell biology research is presented. The examples presented here are the following: estimation of (i) cytoplasmic viscosity, (ii) cytoplasmic pH, and (iii) membrane fluidity and heterogeneity. We use a variety of fluorescence probes to monitor the above in various cell types. The following fluorescence parameters are used in this work: fluorescence lifetime, rotational reorientation time, and fluorescence lifetime distribution by the maximum entropy method. We show how one could address and provide answers to specific questions even in a complex system such as a living cell.

THE living cell is a very complex machinery wherein a variety of physical and chemical processes occur. Our current knowledge on many of these processes has accrued from *in vitro* studies in isolated systems. Although such reductionist approaches are very powerful and have yielded a wealth of information on cell physiology, *in vivo* studies on living cells have their own attraction over *in vitro* studies which, in some cases, fail to provide accurate descriptions. These failures, in many cases, are due to the nonapplicability of *in vitro* studies to the processes occurring in intact systems. These processes could be environment-sensitive and reproduction of these environmental conditions in the *in vitro* studies is plagued by the lack of knowledge on the exact conditions applicable for the process of interest. Hence it becomes important either to observe the process of interest *in vivo* or to get the information on the environmental conditions relevant for that process.

Several physical techniques have been devised in the past decades for studying cellular processes *in vivo*. Fluorescence microscopy[1] is one of such techniques extensively used in biomedical research. This technique allows one to study *living* cells and tissues in culture. Also it offers high sensitivity and selectivity arising from specific fluorescence labeling of the system of interest. The technique of fluorescence microscopy itself is not new and has been in use for a long time. Mapping the system of interest (e.g. a specific protein) in a cell suspension has been the main use so far. Occasionally quantitative measurements have been made to estimate either the amount of a particular material present in different locations or the state of the environment and local dynamics[2,3]. For such measurements the fluorescence intensity has been the only parameter widely used. Although such steady state fluorescence microscopy has been proved to be extremely useful in many studies, its quantitative capability suffers due to some drawbacks. Light scattering due to cellular suspensions, background fluorescence and variations in the path length of the observed sample volume often make the measurement of fluorescence intensity unreliable.

Time-resolved fluorescence overcomes many of the problems associated with fluorescence intensity measurements[4,5]. The parameters obtainable from time-resolved measurements are (i) Fluorescence lifetime, (ii) Rotational correlation time, (iii) Lifetime-resolved spectra, and (iv) Number of fluorophore populations (having distinguishable lifetimes) and population heterogeneity. Judicial use of these parameters with appropriate models would offer powerful insight into the dynamics of molecular systems. Thus time-resolved fluorescence techniques when combined with a fluorescence microscope offer the capability to address interesting problems in cell physiology. A striking disadvantage of time-resolved techniques over steady-state measurements, in general, is the loss of signal intensity which occurs during time resolution. However, the use of high sensitivity detection systems largely alleviates these problems. A word of caution is in order at this juncture. One could question the appropriateness of applying rigorous physicochemical methodologies, evolved for well-defined simple systems, to complex systems such as living cells. It is to be noted that such applications are quite appropriate provided the problems addressed are well defined and devoid of any ambiguities.

In the work described here, we have addressed the following three aspects of living cells and have given answers through the use of time-resolved fluorescence microscopy. These are (i) cytoplasmic viscosity, (ii) cytoplasmic pH, and (iii) cell membrane fluidity.

Time-resolved fluorescence microscopy

The technique of time-resolved fluorescence microscopy is powerful in studying various aspects of cell structure, dynamics and function (refs 6–8 and references cited therein). The basic experimental set-up which is a combination of a time-resolved fluorescence spectrometer and a fluorescence microscope offers the capability to perform dynamic fluorescence measurements with a spatial resolution of a micrometer.

The experimental set-up used in the present study is shown in Figure 1 (ref. 6). An inverted epifluorescence microscope (Nikon, Diaphot 300) was optically modified in order to steer the dye laser pulses into the optical path of the microscope. A CW mode-locked frequency-doubled Nd–YAG laser-driven dye (Rhodamine 6G) laser which generates 4–10 ps pulses was used[9,10]. The laser beam was guided to the objective lens (40×, NA 0.55, Nikon or 40×, NA 0.65 Leitz) by a dichoric mirror (610 nm, XF43 of Omega Optical Co, USA) and focused on to the cell suspension. The diameter of the focused beam was estimated to be 1.2 ± 0.2 μm. The fluorescence which was collected by the same objective lens was passed through a cut-off filter, a Glan–Taylor polarizer and a pin hole assembly (placed at the image plane) and detected by a thermoelectrically cooled (Products for Research, USA) fast response photomultiplier (XP2020Q). Time resolution of the fluorescence signal was obtained by the time-correlated single-photon-counting experimental setup described elsewhere[9,10].

The entire set-up of time-resolved fluorescence microscope was extensively tested with several standard samples whose fluorescence lifetime, rotational correlation time and initial anisotropy could be accurately estimated by the microscope-based set-up. Further tests with focused (~ 1 μm) laser beams on standard homogeneous samples showed that we could perform reliable time-resolved fluorescence measurements on a minimum of ~ 100 fluorophore molecules. This, and the fact that a temporal resolution of ~ 50 ps and a spatial resolution of ~ 1 μm is obtainable, makes this set-up quite powerful.

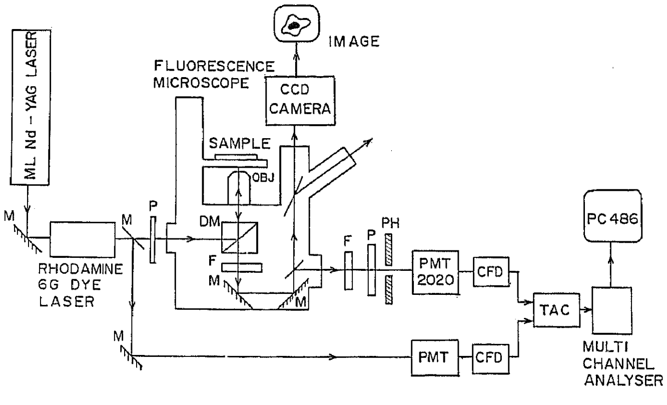


Figure 1. Schematic diagram of the experimental set- up. ML Nd–YAG LASER, mode-locked Nd-YAG laser; M, mirror; P, polarizer; DM, dichroic mirror; OBJ, objective lens; F, filters; PH, pin-hole assembly, PMT 2020, signal photomultiplier XP2020Q; PMT, start signal photomultiplier; CFD, constant-fraction discriminator; TAC, time-to-amplitude convertor; PC 486, IBM PC 486 computer.

Cytoplasmic viscosity

There have been lively and controversial discussions on the physical state of water inside living cells[11,12]. The observation of water bound to macromolecular surfaces[13] and the fact that the intracellular medium is crowded with cytoskeletal networks, macromolecules and small solutes[14,15] raises the possibility of altered properties of intracellular water when compared to bulk water. There have been several experimental attempts in answering this question. Early experiments by using mainly magnetic resonance methods[16,17] had indicated significantly high value (2–8 times that of bulk water) for the viscosity of intracellular water. More recently, fluorescence microscopy techniques have been applied in characterizing the fluid-phase viscosity of cell interior. Several techniques such as fluorescence depolarization of small molecule probes[18,19], fluorescence recovery after photobleaching[20] and steady state fluorescence intensity (viscosity-sensitive quantum yield)[21] have been used. The general conclusion from these studies is that the fluid-phase viscosity of cytoplasm is very close (~ 1.2 cP) to that of bulk water (1.0 cP). However, the possibility of spatial heterogeneity of cytoplasmic viscosity has not been addressed in sufficient detail. Phase separation in cytoplasm leading to 'compartmentation'[22–24] could lead to spatial heterogeneity of cytoplasmic viscosity. Although spatial organization in terms of cytoskeletal network is quite expected for cellular functions such as cell division, motility and maintenance of cell shape, the relevance of spatial heterogeneity of viscosity is less clear.

The dynamics of some of the chemical and transport processes occurring inside a living cell could be dependent on the viscosity of cytoplasm apart from other (rate-limiting) process. In principle, the cytoplasmic viscosity could be controlled by at least two factors: (i) structuring of solvent water by the presence of high concentration of macromolecules[25] and (ii) the presence of high concentration of macromolecules and small molecules such as metabolites which could directly control the viscosity. Since the composition of cytoplasm is highly dependent on cell type[26] and the location within the cytoplasm, the fluid-phase viscosity of cytoplasm is also expected to be dependent on both the cell type and the location.

We have estimated the cytoplasmic viscosity of a number of cell types by monitoring the rotational diffusion of a fluorescence probe Kiton Red (Figure 2). The parameter used here is the rotational correlation time τ_r. Cells are loaded in Kiton Red and probed with a spatial resolution of ~ 1 μm using the focused laser beam[6]. Figure 3 shows typical decay curves of total fluorescence intensity (*b*) and fluorescence anisotropy (*c*) of Kiton Red in a living cell (*a*). The intensity decay kinetics could be nicely fitted to a sum of two exponentials.

$$I(t) = \alpha_f \exp(-t/\tau_f) + \alpha_b \exp(-t/\tau_b), \quad (1)$$

where $I(t)$ is the intensity at time t, α is the amplitude and τ is the fluorescence lifetime. $\alpha_f + \alpha_b = 1$. Analyses showed that τ_f (1.5 ns) and τ_b (3 ns) represent the lifetimes of Kiton Red 'free' and bound to macromolecules in the cell respectively[6]. The fraction (α_b) of the bound population was < 30% in all the cell types studied. The rotational dynamics of the 'free' population of Kiton Red was used in the estimation of cytoplasmic viscosity.

Decay of fluorescence anisotropy (Figure 3 *c*) shows 'dip-and-rise' pattern in most of the cells and in various model systems such as giant single liposomes. The dip-and-rise is a consequence of the two population models[27]. The anisotropy r at time t is given by

$$r(t) = f_f(t)\, r_f(t) + f_b(t)\, r_b(t), \quad (2)$$

where $f_f(t)$ and $f_b(t)$ are the fractions of the emitted photons corresponding to the free and bound Kiton Red at time t respectively. $r_f(t)$ and $r_b(t)$ are the anisotropy decay functions of the free and bound probes respectively.

$$r_f(t) = r_{0f} \exp(-t/\tau_{rf}), \quad (3)$$

$$r_b(t) = r_{0b}(\beta_{b1} \exp(-t/\tau_{rb1}) + \beta_{b2} \exp(-t/\tau_{rb2})). \quad (4)$$

The anisotropy decay ($r_f(t)$) of free Kiton Red followed a single exponential (as observed in bulk solutions) and that of the bound Kiton Red ($r_b(t)$) followed a sum of at least two exponentials. Of the two correlation times of bound Kiton Red (τ_{rb1} and τ_{rb2}) one could correspond to local motion and the other could represent the global motional dynamics of the macromolecule to which the Kiton Red is bound. The fraction $f_f(t)$ is given by

$$f_f(t) = \frac{\alpha_f \exp(-t/\tau_f)}{\alpha_f \exp(-t/\tau_f) + \alpha_b \exp(-t/\tau_b)}. \quad (5)$$

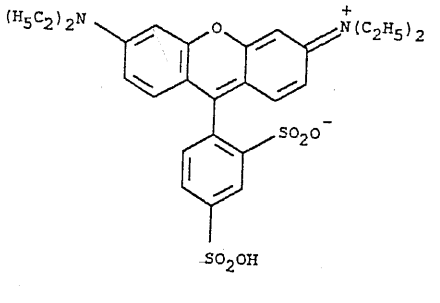
The anisotropy decays were analysed according to this model (see the smooth line fit in Figure 3 *c*) and the results are shown in Table 1. τ_{rf} has the information on the cytoplasmic viscosity.

For a dipolar molecule such as Kiton Red rotating in a polar solvent, the rotational correlation time τ_r is given by[28–30]

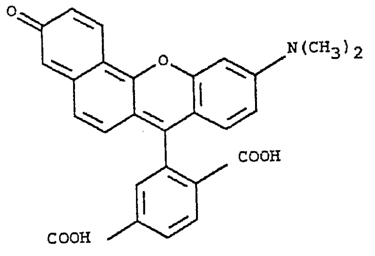
$$\tau_r = \tau_h + \tau_{DF}$$

$$\tau_r = \frac{\eta V f C}{kT} + \frac{4\pi\mu^2}{3VkT} \frac{(\varepsilon - 1)}{(2\varepsilon + 1)^2} \tau_D, \quad (6)$$

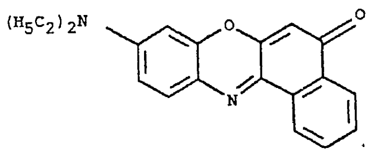
where τ_h and τ_{DF} are the contributions due to hydro-



Kiton Red



SNARF -1



Nile Red

Figure 2. Fluorescence probes used in this work. The water-soluble Kiton Red was used for the estimation of cytoplasmic viscosity; SNARF-1 is a pH probe; Nile Red partitions into membranes and report their dynamics.

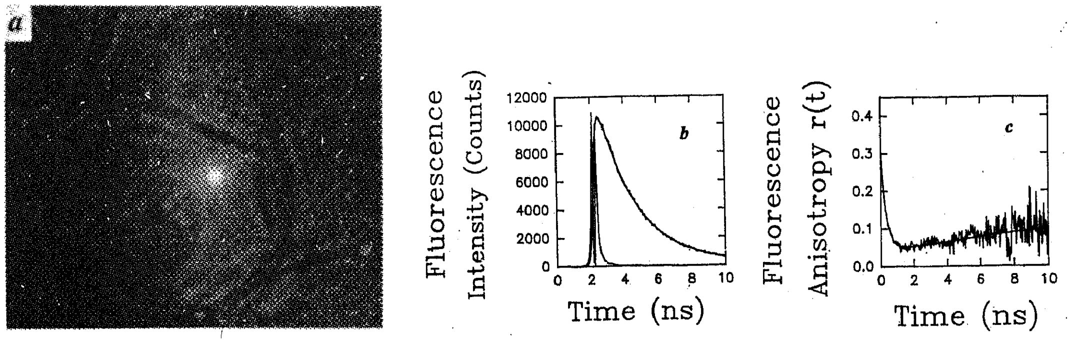


Figure 3. *a*, A single cell of *Tradescantia* under observation. The focused laser spot is also seen. The decay of fluorescence intensity (*b*) and fluorescence anisotropy (*c*) of Kiton Red corresponding to the sample (*a*) is shown. The smooth lines in *b* and *c* are the fits according to the models (see text).

dynamic (Stokes–Einstein–Debye–Perrin) and dielectric friction respectively. η is the solvent viscosity, V is the molecular volume of the probe, f is the molecular shape factor ($f \geq 1$), C is a factor ($0 \leq C \leq 1$) describing hydrodynamic boundary conditions, μ is the dipole moment of the probe molecule, ε and τ_D represent the dielectric constant and Debye dielectric relaxation time of the solvent respectively. It has been shown that $\tau_h \gg \tau_{DF}$ in aqueous solvents[29] and hence the change in τ_{rf} could be used in getting information on η. η values estimated in this way are also given in Table 1. (A value of 1.0 cP for η in water was used as the standard.)

The following picture emerges from these data: (i) the cytoplasmic viscosity is not very far from that of bulk water for all the cells studied; (ii) the average value of cytoplasmic viscosity is larger in case of the plant cells when compared to those of the animal cells studied here; (iii) the positional variation in the value of cytoplasmic viscosity is larger in the case of the plant cells when compared to the animal cells; 3-fold variation was seen in some cases; (iv) the average value of viscosity is closer to the lower bound of the extent of spatial variation of viscosity in all the cells studied showing the dominance of low-viscosity region in the total cell volume; (v) cytoplasmic viscosity inside the tumor cell Sarcoma-180 is not different from the normal mammalian cells studied here; (vi) η values were higher near the plasma membrane or near the cell–cell junction when compared to the cell interior; (vii) η values estimated in either cytoplasm or nucleoplasm were very similar to each other, and (viii) η values were very similar in either adherent monolayer cells (such as 3T3 and CHO) or suspensions such as human mononuclear cells.

Our observation of cell type dependence and spatial location dependence of the cytoplasmic viscosity could have the origin in the variation in one or more of the following conditions within the cells: (i) the level of structured water, (ii) the concentration of small and macro molecules, (iii) the hydrodynamic shape (compact or extended structure) of the molecules present, and (iv) physical restrictions such as proximity of membrane and cytoskeletal network.

What is the relevance of these observations in cell biology? Firstly, our finding of higher value of average cytoplasmic viscosity in the plant cells when compared to animal cells could be the result of either higher concentration of solutes or larger fraction of molecules which have higher influence on the solvent viscosity. These could be either small molecules or macromolecules in unstructured open chain conformation (see above). Secondly, the spatial location dependence of viscosity in some of the cells studied (Table 1) could be due to observed variation in the solute composition apart from factors such as spatial restriction. Cell cytoplasm is a crowded milieu[14,15] and is composed of a variety of molecules of various sizes and shapes. The present finding of spatial dependence of viscosity supports the hypothesis of phase separation in cytoplasm[22–24]. The proposed phase separation which could result as a consequence of segregation of incompatible macromolecules could be important in many cell physiological functions such as channelizing metabolites and macromolecules into specific organelles. Spatial dependence of viscosity would imply spatial location dependence of rates of reactions which are controlled by diffusion. Modulation of reaction rates across the dimension of the cell is an attractive and unexplored area in cell biology. The estima-

Table 1. Typical parameters associated with the decay of fluorescence anisotropy of Kiton Red inside various cells

	Rotational correlation time (ns)[a]			Amplitudes				Viscosity η (cP)[b]		
	Free	Bound		Free	Bound					
Cell type	τ_{rf}	τ_{rb1}	τ_{rb2}	β_f	β_{b1}	β_{b2}	χ^2	Typical	Range	Average
Mammalian cells										
Chinese hamster ovary cells	0.22 ± 0.02	6.0	> 20	0.86	0.08	0.06	1.01	1.1	1.0–1.4	1.1
Swiss 3T3 fibroblast	0.22 ± 0.02	10.0	> 20	0.86	0.10	0.04	1.01	1.1	1.0–1.4	1.1
Human mononuclear cell	0.24 ± 0.03	10.0	> 20	0.90	0.01	0.10	1.10	1.2	1.0–1.5	1.1
Sarcoma-180 tumour cell	0.22 ± 0.02	3.5	> 20	0.51	0.29	0.20	1.10	1.1	1.1–1.4	1.2
Plant cells										
Stamen hair cells of *Tradescantia*[c]	0.24 ± 0.02	3.0	> 20	0.59	0.25	0.16	1.10	1.2	1.2–3.0	1.5
	0.35 ± 0.04	2.0	> 20	0.63	0.19	0.18	1.10	1.7		
	0.45 ± 0.06	3.5	> 20	0.50	0.30	0.20	1.10	2.3		
Digitalis lanata	0.35 ± 0.03	13.0	> 20	0.58	0.21	0.21	0.99	1.8	1.1–3.7	1.3
Guard mother cells of *Allium cepa*	0.30 ± 0.02	7.0	> 20	0.78	0.15	0.07	1.01	1.5	1.1–3.5	1.3

[a] The error in the parameters is about 10%.

[b] η was calculated by using the equation $\tau_{rf} = \eta V/kT$ and the value of 1.0 cP for the η in buffer. 'Typical' viscosity values refer to the typical data given for each cell type. Range of viscosity corresponds to the extent of variation observed during many single spot measurements either on a same cell or on many cells. Average viscosity refers to total observations on a collection of cells without spatial resolution.

[c] The three entries correspond to three different positions within a single cell. The second and third entries correspond to positions very close to cell–cell junctions.

tion of cytoplasmic viscosity is one example which shows that one could address and answer well-defined questions even in a complex system such as a living cell.

Modelling cytoplasmic viscosity

In order to check whether the observed high viscosity in some region of the cells is due to any physical restriction, the rotational diffusion of Kiton Red in the inner aqueous phase of sonicated lipid vesicles (300 Å dia)[31] was measured. Analysis showed that the solvent viscosity in the inner aqueous compartment is ~ 2–3 cP which is significantly higher when compared to the bulk viscosity. This suggests that the higher value of viscosity observed in some part of the cells such as locations very close to membranes or at junctions between cells (Table 1, *Tradescantia* cells) could be due to physical restrictions dictating the apparent viscosity.

Another important aspect in modelling the cytoplasmic viscosity is the effect of macro molecules and small molecules which could be present in high concentrations. The concentration of proteins and nucleic acids in cytoplasm is as high as 10–20% by weight[14,15,32]. We observed that the rotational diffusion of free Kiton Red was retarded by the presence of either small or large molecules[6]. The estimated solvent viscosity was in the range of 1.1 cP to 1.9 cP at 5% (w/v) concentration of the additives. A few specific comments could be made on these observations: (i) The apparent solvent viscosity increased (from 1.2 cP to 1.9 cP) with the increase in the average molecular weight of the polyethylene glycol from 200 to 10,000 and (ii) The viscosity depended also on factors other than the molecular weight of the additive. For example, polyglutamic acid (mol. wt 14,000) had almost no effect on the viscosity whereas the observed viscosity was 1.9 cP in the presence of either polylysine (mol. wt 19,000) or polyethylene glycol (mol. wt 10,000). The overall picture which emerges from these data is that the hydrodynamic shape of the macromolecule dictates the apparent solvent viscosity sensed by small molecule probes such as Kiton Red.

Estimation of intracellular pH

Intracellular pH is implicated in the survival, proliferation and signalling in cells[33]. Optical measurement of intracellular pH scores over other methods of pH estimation in two respects: (i) facile measurement of real time variation of intracellular pH, and (ii) the capability to map the spatial variation of pH within a single cell with the help of fluorescence microscope. A variety of fluorescent pH indicators have been developed specially for intracellular pH measurements[34]. Although fluorescence intensity is the most convenient parameter for measurements, the convenience is offset by various uncertainties in the estimation of pH from fluorescence intensity. The measured intensity would depend upon the following factors also apart from the pH: (a) concentration of the probe, (b) path length of the observation along the *z*-axis of the microscope optics, (c) photobleaching of the probe, (d) scattering by the specimen, and (e) binding of probe to macromolecules and membranes. In order to overcome these drawbacks, dual emission wavelength probes were recently introduced. In these probes, the emission intensity at one of the wavelengths is independent of pH (isoemissive wavelength) and that at another wavelength depends on pH. Hence, estimation of pH from the ratio of intensities at the two wavelengths will correct for the first three of the causes listed above. However even this nontrivial ratiometric procedure cannot correct for probe binding. Binding of probes to macromolecules and membranes could result in (i) significant changes in the emission spectrum and quantum yield and (ii) apparent shift in the acid-base equilibrium of the probe. These perturbations would cause significant levels of error in the estimation of pH.

Measurement of fluorescence lifetime could be an alternative approach for the estimation of pH[35,36]. The principle of lifetime-based pH sensing lies in the estimation of relative amplitudes of the lifetime components of the protonated and deprotonated forms of the probe which have distinctly different lifetimes. The main advantage of lifetime-based sensing is the absence of complications due to change in the path length of observation and the presence of light scattering and photobleaching. We have evaluated, using time-resolved fluorescence microscopy the performance of the dual emission pH probe carboxy SNARF-1 (Figure 2) which is being widely used in cell biology (e.g. refs 37, 38).

The fluorescence decay curves of carboxy SNARF-1 in buffers of various pH values fit to a sum of two exponentials of time constants 0.5 ns and 1.5 ns representing the protonated and deprotonated forms of carboxy SNARF-1 respectively[39]. Plots of the amplitudes associated with the two lifetimes indicated a pK of ~ 6.7 similar to the observations with the fluorescence intensity[39]. The mean lifetime τ_m $(= \Sigma_i \alpha_i \tau_i)$, which is proportional to the steady-state fluorescence intensity, also showed a similar titration with pH.

Fluorescence decay of carboxy SNARF-1 loaded into a variety of cell types showed that, unlike in the case of buffers (see above), the decays could not be fitted to a sum of two exponentials. A sum of three exponentials could fit the decay curves quite satisfactorily. Free fits of the decay curves gave the following three lifetimes in most of the cases: 0.5–0.6 ns, ~ 1.5 ns and 3–4 ns (Table 2). In some cases a fourth lifetime component of ~ 0.1 ns was also necessary to fit the decay curves

(Table 2). It is likely that the lifetime components 0.5–0.6 ns and 1.5 ns represent the protonated and deprotonated forms of unbound SNARF-1 respectively and the other two lifetimes could be due to bound populations of SNARF-1.

The fractions of the bound populations was quite small $((\alpha_3+\alpha_4)\leq 0.2)$ in all the cases, indicating that the majority of the probe was in the free form inside the cells. However significant levels of errors in the estimation of pH could arise if these bound populations are not taken into account, as shown below. Standard curves obtained in buffer solutions showed that the pH values estimated either from the mean lifetime (τ_m) or from the fractional amplitudes are identical[39]. In contrast, in all the cell lines studied, the pH values estimated either from τ_m or from the normalized amplitude of either the protonated (~ 0.5 ns) or deprotonated (~ 1.5 ns) forms of free carboxy SNARF-1 differ substantially from each other (Table 2). In these estimations, the amplitude of the protonated form (for example) was renormalized as the fraction of the total amplitudes corresponding to the protonated and deprotonated free forms (Table 2). It can be seen that, in most of the cases, the pH values estimated by τ_m is about 0.1–1.0 pH unit higher than that estimated from the renormalized amplitudes of the free probe. The origin of this difference lies in the higher value (~ 3 ns) of the lifetime of the predominant population of bound probe when compared to those of the free forms. In case where a fourth lifetime component (τ_4 ~ 0.1 ns) was observed, the pH value estimated by τ_m is lower than that estimated by the renormalized amplitude. Although the difference of 0.1–1.0 pH unit seen in our pH estimations based on either τ_m or normalized amplitudes may be considered as relatively small, such differences could turn out as significant in situations where the level of probe binding is even higher. Also such differences arising from probe binding could be incorrectly interpreted as due to pH gradients within a cell.

In summary, we have shown that the binding of the probe inside the cells could lead to significant levels of error in pH estimations. Separation of the free populations (from the bound probe) using the time resolved fluorescence has been established as a reliable method of intracellular pH estimation.

Membrane structure and dynamics

Apart from providing boundaries of living cells and of their organelles, biological membranes control many of the functions of the cell through their physical state. Hence a knowledge of their physical state is important for a complete description of cell physiology. Although the physical state of pure lipid bilayers has been characterized in terms of gel and liquid crystalline phases, the presence of microdomain structures suggested in the case of mixed lipid membranes[40,41] and cell membranes[42,43] could also control their physical state. There are several molecular mechanisms for microdomain structures: (i) segregation of lipids in mixed lipid membranes

Table 2. Parameters obtained from the analysis of fluorescence intensity decays of carboxy SNARF-1 in various cells and intracellular pH values estimated by either the mean lifetimes or the normalized amplitude of the unbound protonated probe

	Lifetimes (ns)[a]				Amplitudes				Mean lifetime τ_m (ns)	Normalized amplitude $\alpha_1/(\alpha_1+\alpha_2)$	pH estimated by	
Cell type	τ_1	τ_2	τ_3	τ_4	α_1	α_2	α_3	α_4			τ_m	$\alpha_1/(\alpha_1+\alpha_2)$
Swiss 3T3 fibroblast[b]	0.56	1.45	3.34	–	0.34	0.58	0.08	–	1.30	0.37	7.4	7.0
	0.64	1.45	3.40	–	0.41	0.54	0.05	–	1.22	0.43	7.2	6.9
	0.58	1.45	3.39	–	0.41	0.53	0.06	–	1.21	0.44	7.2	6.9
	0.65	1.45	2.98	–	0.47	0.52	0.01	–	1.09	0.47	6.9	6.8
	0.56	1.42	3.20	–	0.33	0.58	0.09	–	1.31	0.42	7.5	6.9
	0.56	1.48	3.23	–	0.27	0.61	0.12	–	1.40	0.30	8.0	7.2
Chinese hamster ovary cells	0.60	1.45	4.31	–	0.36	0.62	0.02	–	1.20	0.37	7.1	7.0
	0.50	1.57	3.73	–	0.37	0.50	0.13	–	1.46	0.42	9.3	6.9
	0.55	1.40	3.20	0.10	0.15	0.24	0.06	0.55	0.67	0.38	5.8	7.0
Human mononuclear cells	0.55	1.42	3.88	–	0.50	0.42	0.08	–	1.18	0.55	7.1	6.6
	0.55	1.42	3.20	–	0.39	0.51	0.10	–	1.26	0.43	7.3	6.9
	0.65	1.54	4.23	0.11	0.27	0.29	0.04	0.40	0.81	0.48	6.2	6.8
Guard mother cells of *Allium cepa*	0.60	1.40	3.70	–	0.46	0.49	0.05	–	1.15	0.48	7.0	6.8
	0.60	1.40	3.70	–	0.55	0.49	0.06	–	1.24	0.41	7.2	7.0
	0.49	1.52	3.71	–	0.40	0.44	0.16	–	1.46	0.48	9.3	6.8
Tumour cell sarcoma-180	0.49	1.42	4.00	–	0.33	0.57	0.10	–	1.37	0.37	7.7	7.0
	0.49	1.42	4.46	–	0.36	0.59	0.05	–	1.24	0.38	7.2	7.0
	0.49	1.42	4.00	–	0.33	0.57	0.10	–	1.36	0.37	7.6	7.0

[a] Chi-square values were between 1.0 and 1.1 for all the data.

[b] The first three entries correspond to three different locations within a single 3T3 cell. Subsequent entries correspond to different single cells.

induced by immiscibility of the components, (ii) binding of Ca^{2+} and positively charged peripheral proteins to segregated negatively charged lipids, and (iii) coexistence of gel and liquid crystalline phases. Apart from such microdomains, special structures such as lipidic cubic phases[44,45] and structures formed by non-bilayer forming lipids such as phosphatidylethanolamine have been suggested to have functional roles in living systems[46,47]. Thus a general description in which the membrane function is controlled by the overall membrane fluidity[48] and local specific structures such as microdomains and non-bilayer structures is quite attractive.

Several physical techniques have been used in the characterization of membrane fluidity, membrane microdomains and non-bilayer structures. Of these, only a few are suited for studies on single living cells. These include optical techniques based on fluorescence microscope. A large variety of fluorescence probes have been developed for studying the structure and dynamics of membranes[49]. Although fluorescence depolarization[18,19] and fluorescence photobleaching recovery[50] are the commonly used methods, there are several other fluorescence-based techniques which could be applied to single living cells.

Fluorescence lifetime distribution of membrane probes

Fluorescence lifetime distribution of membrane probes could be a powerful method in characterizing the membrane structure and dynamics through the heterogeneity of population of the probe. The fluorescence decay kinetics of probes distributed in complex systems generally show a considerable level of heterogeneity. It is reasonable to assume that, in the absence of evidence of the number of population of the probe, the probe is distributed into a very large number of populations whose properties could vary from each other. In such situations, fitting the observed decay kinetics to a distribution of lifetimes is more rational when compared to fitting them to a sum of a few (≤ 4) discrete exponentials. Several functional forms such as Lorentzian or Gaussian distributions have been used in the past in several situations such as proteins[51,52] and membranes[53,54]. However it should be clearly pointed out that there is no physical rationale based on any model for the use of the functional forms mentioned above. In contrast, fitting the fluorescence decay kinetics in such complex systems to a distribution of lifetimes based on the maximum entropy method (MEM) is quite attractive[55–57].

The fluorescence intensity ($I(t)$) decay kinetics could be represented by the general expression

$$I(t) = \int_0^\infty \alpha(\tau) \exp(-t/\tau)\ \mathrm{d}(\tau)\,, \qquad (7)$$

where τ is the fluorescence lifetime and the function $\alpha(\tau)$ represents the distribution of lifetimes. In the case of discrete exponential fits, eq. (7) reduces to

$$I(t) = \sum_i \alpha_i \exp(-t/\tau_i) \qquad i = 1\text{–}4, \qquad (8)$$

and in the case of Lorentzian fits, $\alpha(\tau)$ is represented by an Lorentzian function.

The MEM (refs 55–57) starts with the assumption that the functional forms of $\alpha(\tau)$ is unknown. MEM begins with an initial condition in which the lifetimes are distributed in a particular range (say 10 ps to 10 ns), all having equal probability (amplitude). The range is covered by N exponentials ($N = 150$) with τ values equally spaced in the $\log \tau$ space. For this distribution the entropy S (Shannon–Jaynes entropy) and the reduced χ^2 are calculated;

$$S = -\sum \alpha_i \log \alpha_i\,. \qquad (9)$$

Data analysis consists of modifying α_i in each iteration such that minimization of χ^2 and maximization of S occurs. Procedures for the modification of α_i in each iteration are given elsewhere[55–57]. For a particular value of χ^2 there will be many possible distributions of α_i; MEM identifies that distribution for which S is maximum. This method will not give structures in the distribution unless warranted by the data. The analysis is terminated if $\chi^2 \leq 1.0$ or if subsequent iterations have negligible effect on the values of χ^2, S and the α_i profile.

MEM gives a continuous distribution of lifetimes as the required solution without assuming any model or mathematical function. The distribution given by MEM is a general one which includes also the solution obtained by a discrete multiexponential analysis. If the decay is truly a sum of N exponentials (representing N populations), then the MEM analysis would generate a distribution with N peaks provided the signal to noise ratio in the data is adequate to resolve the peaks[57].

There are several features which are apparent in lifetime distributions obtained by MEM. They are (i) the number of peaks, (ii) the width of the distribution, and (iii) the shape of the distribution. The width of the peaks is dependent on the signal to noise ratio; the width decreases with the increase in peak counts and levels off at high peak counts[58]. This residual width which is likely to represent the true width of the system could be ascribed to the heterogeneity of the probe. In the present work, we use the width of the distribution associated with the membrane probe Nile Red in gaining insight into heterogeneity in the population and hence the dynamics of membranes. Nile Red is commonly used to label membranes in cells[58,59] and the fluorescence decay kinetics of Nile Red is very sensitive to the environment[60].

Fluorescence lifetime distribution in pure lipid membranes

Figure 4 shows the distribution of fluorescence lifetime of Nile Red in either in DMPC or DPPC vesicle membranes at 35°C. The gel–liquid crystalline phase transition temperatures of DMPC and DPPC are 25°C and 42°C respectively[61]. Hence DMPC and DPPC are expected to be in the liquid crystalline phase and the gel phase respectively at 35°C. The most striking feature of these distributions is the difference in the width between DMPC and DPPC membranes. The width observed in the case of DPPC was about 3–4 times larger when compared to that observed in the case of DMPC (Table 3 and Figure 5).

Figure 5 shows the temperature dependence of width in the case of Nile Red in DMPC, DPPC, egg PC and soybean phospholipid vesicles. It can be seen that the width tracks accurately the physical state of the lipids, with transition temperature around 25°C and 42°C in the case of DMPC and DPPC respectively. In contrast, the width did not show any transition in the cases of egg PC and soybean phospholipid as expected. One could have several models for explaining the variation of width in pure lipid membranes. In the membrane, Nile Red molecules could be distributed into various orientations and environments each having an individual lifetime. In the gel phase, the translational and rotational mobility of Nile Red is likely to be slower than the decay time. This would result in a continuous distribution of lifetimes represented by a broad gaussian as observed in the case of membranes below the phase transition temperature (Figures 4 *b* and 5). In contrast, in the liquid crystalline state, Nile Red molecules could be highly dynamic and experiences an average environment during the lifetime of the excited state. This would result in narrowing down of the distribution since the average lifetime of all the Nile Red molecules become similar to each other. The narrower distribution obtained above the phase transition temperature (Figures 4 *a* and 5) would fit with this explanation.

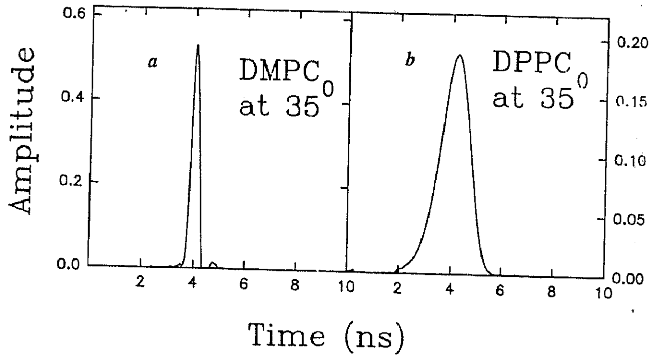


Figure 4. Fluorescence lifetime distribution (analysed by the maximum entropy method) of Nile Red in DMPC (dimyristoyl phosphatidyl choline) and DPPC (dipalmitoyl phosphatidyl choline) vesicles at 35°C. At this temperature, DMPC is in liquid crystalline phase and DPPC is in gel phase.

Could we gain insight into the various populations of Nile Red responsible for the continuous distribution of lifetimes? An attractive model is the orientational heterogeneity wherein there is a continuous distribution of θ, the angle between the emission dipole of the probe and the normal to the surface of the bilayer membrane. The radiative rate (k_r) is a function of θ and the refractive index of the membrane (n_m) and the aqueous phase (n_0) (ref. 62).

$$k_r = (\tfrac{3}{2}\ r \sin^2\theta)\, n_0 + (\tfrac{3}{2}\ r n_m^{-4} \cos^2\theta)\, n_0^5, \qquad (10)$$

Table 3. Parameters obtained by the maximum entropy method analysis of fluorescence intensity decay of Nile Red in lipid membranes

Composition of vesicles[a]	Width of lifetime distribution (ns)	Peak position (ns)	Order parameter (*S*)
DMPC at 35°C	0.34	3.90	0.45
DPPC at 35°C	1.70	4.22	0.72
DMPC-60% (w/v) sucrose at 35°C	0.27	3.91	0.31
DPPC-60% (w/v) sucrose at 35°C	0.72	4.12	0.79
ePC at 25°C	0.34	3.55	
SPL at 25°C	0.51	3.60	0.51
SPL + 14% cholesterol	1.33	3.85	
SPL + 28% cholesterol	1.83	3.92	0.75
SPL + 57% cholesterol	2.62	4.18	
ePC + 40% (w/w) mellitin	0.87	3.78	
ePC-PA (1 : 1) + 10 μM cytochrome C	0.97	4.07	
SPL + cytochrome oxidase (lipid/protein ratio 20 : 1)	0.97	4.07	
DMPC + 10% (w/w) capric acid	0.70	4.40	

[a] DMPC, dimyristoyl phosphatidyl choline; DPPC, dipalmitoyl phosphatidyl choline; ePC, egg phosphatidyl choline; SPL, soybean phospholipids; PA, phosphatidic acid; cholesterol percentage is by molar ratio.

where r is the combination of several constants[62]. Since $\tau = 1/(k_r + k_{nr})$, where k_{nr} is the combined rate of all the non-radiative processes, the observed lifetime τ will depend upon θ and hence the orientation of the probe with respect to the membrane plane. If the orientation is distributed over a large range of θ, then one would expect a broad distribution of τ. When the refractive index of the membrane (n_m) and the aqueous phase (n_0) are equal to each other, k_r will be independent of θ. It has been shown that $n_m \approx n_0$ at 60% (w/v) sucrose in the aqueous phase[62,63]. The width observed in DPPC membranes in 60% sucrose was significantly smaller when compared to the situation in sucrose-free buffers. Thus the difference in the width in the gel and liquid crystalline phase is very much reduced in the presence of 60% sucrose. These results indicate that the orientational distribution of Nile Red is the major source of larger width in the gel phase.

Lifetime distribution in mixed lipid membranes and lipid–protein membrane

As mentioned earlier, the physical state of cell membranes could also be controlled by microdomain structures which could be created in a variety of ways. Mixtures of incompatible lipids, binding of ions such as Ca^{2+} and some peripheral proteins are some of the causes of formation of membrane domains[40–43]. Distribution of Nile Red into the domain structures, protein–lipid interfaces and other regions of the membrane could increase the heterogeneity and hence the width of the distribution. In such a model, the distribution of τ would arise mainly from the heterogeneity of k_{nr} rather than in k_r. Table 3 lists the width of the distribution obtained in a variety of lipid mixtures and in the presence of peripheral and membrane proteins. It can be seen that cholesterol has a significant influence on the width. The increase in the width and in the position of the peak with the increase in cholesterol content suggests increased rigidity of the membrane with the increase in the level of cholesterol. Alternatively, cholesterol could have created domains in the membrane leading to increased heterogeneity in the environment of the Nile Red. The heterogeneity in τ is caused by the heterogeneity in k_r in former model and by the heterogeneity in k_{nr} in the latter model. The decay kinetics of fluorescence anisotropy of Nile Red supports the first model wherein the membrane shows increased gel character with the increase in cholesterol content. This was revealed in the increase in the order parameter, S ($S = (3 < \cos^2\theta > - 1)/2$) with the increase in the cholesterol content (Table 3). Another perturbation which resulted in a significant increase in the width of lifetime distribution was the presence of membrane proteins such as mellitin and cytochrome oxidase (Table 3). The increase in the width in these

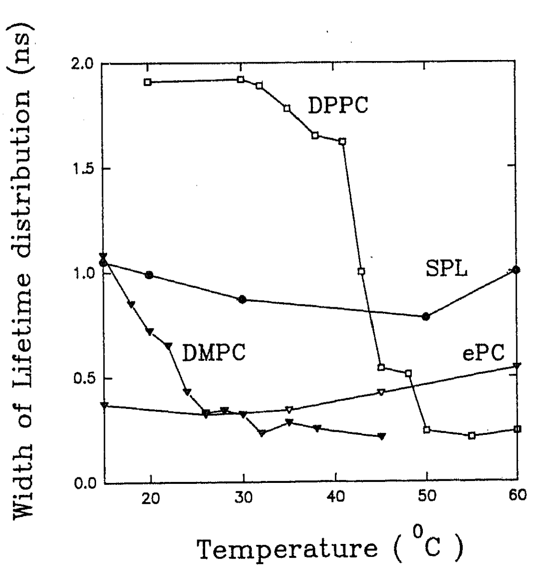


Figure 5. Temperature dependence of the width of lifetime distribution of Nile Red in various membrane vesicles. ePC, egg phosphatidyl choline; SPL, soybean phospholipids.

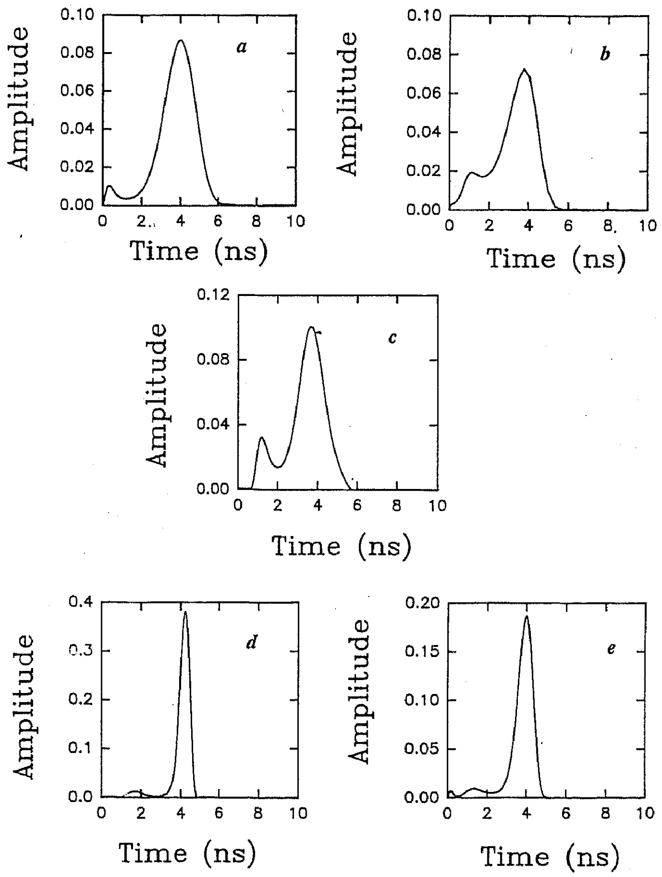


Figure 6. Maximum entropy method distribution of fluorescence lifetime of Nile Red in various cells. ***a***, Chinese hamster ovary cells; ***b***, Human mononuclear cells; ***c***, Swiss 3T3 fibroblast; ***d***, Guard mother cells of *Allium cepa*; ***e***, Stamen hair cells of *Tradescantia*.

Table 4. Parameters obtained by maximum entropy method analysis of fluorescence intensity decay of Nile Red in various cells

	Width of lifetime distribution (major peak) (ns)		Peak position (ns)	
Cell type	Mean	Standard deviation	Mean	Standard deviation
Mammalian cells				
Chinese hamster ovary	1.65	0.64	3.97	0.24
Swiss 3T3 fibroblast	1.73	0.31	4.23	0.20
Human mononuclear cells				
Plasma membrane	1.80	0.45	4.10	0.41
Nuclear membrane	1.02	0.38	3.94	0.24
Plant cells				
Guard mother cells of *Allium cepa*	0.94	0.27	4.07	0.08
Stamen hair cells of *Tradescantia*	0.77	0.41	4.36	0.17

cases is most likely due to the heterogeneity in the environment of Nile Red. Nile Red molecules present either at the protein–lipid interface or bound to the membrane protein would have lifetimes different from the population in the pure lipid region.

Lifetime distribution in cell membranes

The prime objective of this work is the assessment of cell membrane dynamics through lifetime distribution of the fluorescence membrane probe Nile Red. We have shown above that the following features of membranes have significant effect on the width of lifetime distribution: (i) The gel–liquid crystal phase state, (ii) presence of cholesterol and other mixtures of lipids, and (iii) presence of integral membrane proteins. Hence a judicial interpretation of the width of distribution in cell membranes could be attempted in terms of the above aspects.

Figure 6 shows some typical lifetime distributions of Nile Red in membranes of a variety of living cells. It could be seen that apart from the mean peak (at around 3.8 ns) which was seen in the pure lipid membranes (Figure 4), another minor peak (at around 0.6 ns) was also observed in most of the cases. It is likely that the major peak represents Nile Red in membranes and the minor peak corresponds to Nile Red bound to proteins and other macromolecules. Table 4 lists typical values of the width and the position of the major peak. Both these parameters showed significant variation from one cell type to another and the position of observation within a cell. The following conclusions could be drawn from the results shown in Table 4: (i) The width observed in the mammalian cells studied here was significantly larger when compared to those observed in the plant cells; (ii) the variation in the width observed in a single mammalian cells was about 2–3 fold, which is significantly larger than the variation observed in the plant cells, and (iii) the width observed in plasma membranes was larger when compared to that in nuclear membrane in the mammalian cells. As mentioned earlier, several factors could control the width of the lifetime distribution. In the light of the model systems described earlier we could assign the increased width with one or more of the following two factors: (a) increased gel character of the membrane and (b) increased level of microdomain structures in the membrane. The studies on cell membranes presented here are preliminary in nature and more detailed investigations on the cell membrane dynamics are in progress.

Conclusions

We have shown, through a few representative examples, the power of time-resolved fluorescence microscopy in studying dynamic aspects of living cells. We have also shown how one could address and answer pertinent questions even while studying an intact complex system such as a cell. The fluorescence probes used in this work have only broad specificities and hence our questions could not address specific cellular components. Design of specific probes and incorporating them into living cells would allow us to study the dynamics of specific systems and specific organelle locations where a particular system reside. Such designs are limited mainly by the ingenuity of the physical biochemist.

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Global change scenario: Current and future with reference to land cover changes and sustainable agriculture – South and South-East Asian context

Suresh K. Sinha

Though the global change – change in land cover, land use, atmospheric concentration of greenhouse gases, loss of biodiversity and many related processes today–has enormous dimensions, this is the result of changes at micro-level, i.e. the village, small towns and higher order as a result of 'development'. Therefore, the corrective action or mitigation strategy should start at that level. There has been almost no change in forest cover in South and South-East Asia between 1978 and 1993. The important driving forces for land cover change are population, affluence and technology. While the developing countries have been attempting to define poverty, no effort has been made to define affluence, and also the responsibility of the affluent sections of the society towards local, regional and global change. The improved technology of crop production, particularly of wheat and rice, has resulted in saving 160 mha of land since 1961–62 in South and South-East Asia. New problems of sustainability of rice – wheat cropping system, groundwater pollution, reduced emphasis on nutritious grains (coarse grains) and stagnation in research on crop improvement have emerged. The regional cooperation and action points are suggested for meeting the new challenges.

NEARLY fifty years ago, I used to bicycle from my village to the District Headquarters, about 20 miles away. It hardly took a few minutes to get out of the mud houses (except three pucca houses) into the open fields and then into pasture land used for grazing as the village common property. The village common property area was more than the area of the village. On the way to the road, and passing through another village during the monsoon season there were several bushes which were covered by many cucurbits and at least three of them were edible as fruits or vegetables. Today, how I long to see that once again, but most of it is gone and there is hardly any common grazing land and the cattle freely roaming on that piece of land. Gone also are those bushes and cucurbits which we relished. One of them, kakoda, used as a vegetable, is no longer seen in the area – a loss of diversity for the region. Neither did I think those surroundings were gradually changing, nor had I a camera to record them or a perception of change. The population of the village which was around 1500 before 1950, could now boast of more than 4000 despite migration by many including myself to towns and big cities like Delhi.

The village is now spread into almost three times of the area I had known as a child. The population has increased, a metal (pucca) road has come making it possible for buses to ply. There are mostly pucca houses for which bricks came from excavation of agricultural land. The ponds which once were filled with water during the monsoon and provided the fruits of *Trapa bispinosa* and fish, have dried, and for every 10 acres (4 hectare) or less of land there is a diesel pump to lift water, and many other changes have occurred. All this happened so gradually to meet the demands of increasing population and 'development' that one hardly saw what was coming. Now scientifically speaking, there has been a change in land cover and land use. I could also go back to my past and talk of disappearance of small forested land between villages and the pollution of the river Ganga which even today I revere because we jumped into its waters from the boat-bridge to swim everyday in the spring and summer during our examinations. I am now hesitant to enter the waters of the same Ganga at the same place.

Levels of land cover changes

The above description without going into details of

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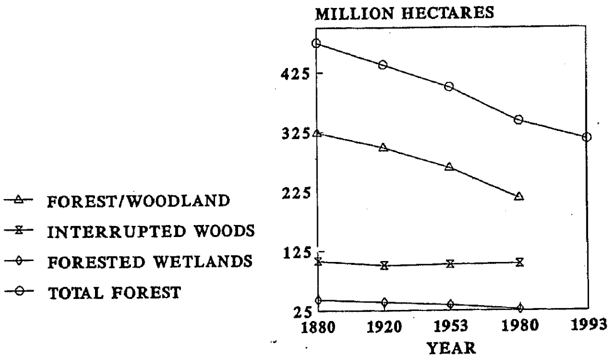
changes in crops and cropping pattern at a village or microlevel provides the process of change in land cover. When taken for a group of villages it will represent changes at a higher level. If we add to this the components of energy, transport and other sectors, we would be addressing the problems of land cover changes at a higher order. These small instances bring out broadly the driving forces for land cover changes. In more specific terms, six components of driving force were identified[1], viz. population, level of affluence, technology, political economy, political structure, and attitudes and values. Though the last three are very important, it is difficult to develop indices to judge their impact. Therefore, a greater effort was put on population, affluence and technology. They were shown to have the following relationship.

Environmental change I = PAT, where P, A and T represent population, affluence and technology respectively. Several authors established a close relationship between population and environment[2,3]. However, it is difficult to identify thresholds of population and the environment in which population resides, because different environments would have different thresholds.

Affluence is considered a major factor for influencing the environment, both at the same location as well as elsewhere. For example if high-quality wood is needed by an affluent country, they buy it from some other country. Therefore, affluence could and does influence environment elsewhere, because of economic reasons. How do we define affluence? In developing countries there is a considerable debate including the international agencies for defining poverty. But possibly the question of affluence has hardly been addressed. The indices for affluence can possibly identify individuals and groups who should pay more attention to their responsibility to the society. In developing countries, a vast population has the primary need of meeting the food demand and therefore they spend 70% or more of their income on items of food. They hardly have money to acquire land for housing and living. But as their income increases, their expenditure on items of food declines and initially expenditure on clothing and housing increases. The expenditure on food articles in most of the developed countries may constitute only 10 to 20% or even less of the total income. Thus expenditure on food items, the area per person for living and expenditure on clothing, recreation and travel could constitute important indicators of affluence. When people spend more on food items, it also is an indication of low level of technology. Accordingly more and more land for agriculture is needed to meet the food demand. This is what happened everywhere in the world leading to deforestation. This happened in 18th and 19th Century in Europe and North America, but the same continued in 20th century, in Asia and Africa. The arrival and improvement in technology for food production changed this pattern. It is likely that technology helped arrest of deforestation in some regions, but it added to environmental problems in others. Since the focus of the present discussion is land cover change and sustainable agriculture, it is addressed to some extent.

Changes in forest cover

The changes in forest cover are important because it is generally felt that in early stages of development deforestation provided land for agriculture and wood for housing and fuel. Since deforestation has possibly contributed CO_2 to the atmosphere (by reducing the sequestering of CO_2 through photosynthesis, and loss of organic carbon from deforested soils), its role in global change has been emphasized. There are historical records to make both estimates and guesstimates for the land cover change involving forests. Richards and Flint[4] described change of land use in South and South-East Asia for the period between 1880 and 1980. There have been many changes in the boundaries of many countries in this region in the past 100 years. Therefore, it is difficult to be accurate in estimates but we have some numbers. However, from 1950 FAO records provide continuity till now though there are some earlier estimates also but they relate to only parts of this region. Richards and Flint[4] included three major categories of forests, viz. forest/woodland, interrupted woods and forested wetlands. The trend for deforestation for South and South-East countries is shown in Figure 1. According to these authors, the forested area in 1980 was 342.6 million hectare, but on the basis of FAO year books, the forested area in 1978 was 318.2 million hectare including that of Nepal and Pakistan which are not included by these authors. This creates a difference of 10% between 1980 and 1993, depending on which numbers we consider. In a period of 110 years



Source: Richards and Flint 1993

Figure 1. Change in forest cover in South and South-East Asia from 1880 to 1993 (million hectares).

the forested area decreased by 35% in South and South-East Asia. Though there are some estimates of contribution of CO_2 to the atmosphere, they have a large element of extrapolation, and sometimes from the non-representative regions.

It is much more important to analyse recent changes in the context of population growth and agriculture. Between 1978 and 1993, a 15-year period, there was a decrease of only 6.0 million hectare forested land being 1.88% of the 1978 forested area. In the meantime, the area under permanent crops such as coconut, oilpalm and rubber increased by 5.7 million hectare (Table 1). If we take these permanent crops as another kind of forest, then virtually there was no deforestation in the last 15 years. The situation in India is also contrary to a general belief. From 1980 till now there has been an increase in dense forest (forest/woodland) and hence in biomass (Table 2). It has been estimated that the carbon-pool in Indian forests increases annually by 8.5 to 12.5 million tons of carbon (TgC).

Thus, there is a need to make better estimates of forest cover and their potential for assimilating atmospheric carbon dioxide. It is possible that deforestation in South and South-East Asia has decreased substantially, and area of dense forests is increasing (Table 3). There are, however, only a few estimates of CO_2 emission from deforested soils. The long term database is required to establish more accurate trends. Some of the models used for this region should be validated and the inputs for different types of forests be improved.

Table 1. Changes in areas of forests and woodland and permanent crops in South and South-East Asia

	Million ha	
	Forests and woodlands	Permanent crops
1978	318.2	23.4
1993	312.2	29.1
	− 6.0	+ 5.7

Table 2a. Decrease in forests woodlands and interrupted forests in South and South-East Asia

Country	Million ha	Change over 1978 (%)
Bangladesh	0.25	13.4
Laos	1.4	10.0
Cambodia	1.58	12.0
Thailand	3.4	19.9
Vietnam	3.8	28.3
Indonesia	7.9	6.62

Based on *FAO Production Year Book*.

Table 2b. Increase in forests woodlands and interrupted forests in South and South East Asia

Country	Million ha	Change over 1978 (%)
Myanmar	0.24	0.73
Nepal	0.1	1.80
India	1.38	2.06
Malaysia	0.56	2.60
Philippines	0.74	5.7
Sri Lanka	0.32	18.0
Bhutan	0.52	20.0
Pakistan	0.71	25.6

Based on *FAO Production Year Book*.

Land cover changes and land-saving technology

Increase in population has an immediate effect on demand for food and housing. In the past 30 years, South and South-East Asian countries have been able to meet their food demand. Countries such as Thailand, Vietnam and to some extent India are emerging exporters of some agricultural commodities. This has occurred through both the increase in area under cultivation as well as increasing productivity of land. The forest cover decreased by 30 million hectare since 1961–63 in this region, and the area for cereal production increased by 30 million hectare. It does not mean that all land for cereal production came from deforestation. Even among cereals there has been a change in land use. For example, the area under coarse grains (including maize) decreased but increased for both rice and wheat. This change resulted in increased cereal production by 235 million tons, a significant part of it coming from the increased productivity. Broadly speaking, the new technology of rice-paddy and wheat production resulted in saving 160 million hectare land coming under cultivation (Table 4). This has often been called the land saving technology[5].

Sustainable agriculture

There are several perceptions of the agriculture and land cover change for South and South-East Asia. It is often

Table 3a. Change in forest area, phytomass C-pool and change in C-pool for the year 1982–1991 (India)

	1982	1991
Change in area (km^2)	24715.98 (dense)	− 25325 (open)
C-pool (mt)	3683–4268	3768–4381
Change in C-pool (mt)	76.27 (low)	113.18 (high)

Dadhwal and Shah[18].

Table 3b. Change in carbon-pool of forests in India

	Tg C (million ton C)
1982	3683–4268
1991	3759–4381
Change	76–113
Annual increase	8.5–12.5

assumed that deforestation has largely been for increasing area for agriculture for meeting the food needs of the growing population. There is a perception, not unjustifiably that agriculture in Asia is becoming unsustainable. Therefore, how would the future demands of food be met? Would there be further deforestation and degradation of natural resources? We first need to define sustainable agriculture, and then try to develop a perception for this region, because identification of problems is only an initial step towards solution which must be the priority now. Nevertheless, the following definitions or statements are important in understanding sustainability. Swaminathan[5] gave the relationship between productivity and other parameters by the equation

$$\text{Productivity} = \frac{\text{Output value}}{\text{Input value}} + \text{Change in environmental stock.}$$

Thus, the concept of various inputs and the value of environment was introduced. He implicitly means that productivity should add or improve the environment. Therefore, it becomes necessary to define environmental stock in terms of either ecology, economics or human well being. For example, the value of water, which is taken for granted, on the banks of big rivers or high rainfall areas may be insignificant, but the same acquires a high value in water-scarcity areas. Therefore, there is a need to put a value to environmental stocks. Maurice Strong[6] stated 'Sustainability means a transition to a wholly new system – one which will permit, in the case of agriculture food production to continue to rise to meet human needs without destroying the underlying basis for supply of nutrients, maintenance of soil conditions. Sustainability is not so hard to define in the field of agriculture as it is in some other fields.'

Kada[7] referred to a simple statement of Wendell Berry, 'A sustainable agriculture doesn't deplete soils or people'. Kada continued 'Over time, an increasing number of researches, farmers, policy makers and organizations worldwide have developed a definition that unifies many diverse elements into a widely adopted comprehensive working definition. Sustainable agriculture is ecologically sound, economically viable, socially just and humane.' Thus, this definition identifies four main factors, but it is likely that in developing countries most of these require far greater clarification than simply abstract concepts.

The Bruntland Commission on Environment and Development Report on Our Common Future[8], defined sustainable development as the one to ensure that it meets the needs of the present without compromising the ability of future generations to meet their own needs.

Thus, the emphasis is on meeting the present needs from the natural resources without impairing them for the future generation. It is not possible to enlarge this discussion to cover many aspects of human development and welfare, but it is best to address a few examples which bring out the dilemma. It has been shown earlier that deforestation has been slowed down considerably in South and South-East Asia in the last 40 years. As stated earlier the decrease from 1978–79 to 1993 in forest area was 6.0 million hectare, 1.88% of the forest area. In the same period, the area under permanent crops increased by 5.7 million hectare. Assuming that the permanent crops such as coconut, oilpalm and rubber represent a different nature of forests, there is no deforestation in this region. More so because there has been no increase in area for food grains.

Table 4. Changes in production, area and rate of total cereals in South and South-East Asia

	Period		
	1961–63	1991–93	Change
Area (mha)	142.2	174.2	30.0
Productivity (mt)	167.2	392.1	234.9
Rate (kg/H)	1179	2251	1072

Additional land requirement 190.2 mha instead of 30.0 mha. Hence saving of 160.2 mha of land.

Rice-based cropping system

Asia as a whole is predominantly a rice producing and consuming region, with about 92% of the total rice production. In South and South-East Asia also, rice remains a major crop but wheat is important in India, Pakistan, Bangladesh and Nepal. These two crops have provided food security in the region. The future of food security of this region is also strongly linked with the sustainability of these crops which have started showing signs of stagnation and deceleration.

There was a very significant change in growth of cereal production from 1961 to 1993. The change in area under cereals grew at a simple growth rate of 0.88% annually but production went up from 167.7 million tons to 392.1 million tons from 1961–63 to 1991–93. This gave a growth rate of 4.46% which outstripped the population growth rate. The latter was 3.23% annually for the same period (Table 5). However, a detailed analysis shows that during the 30-year period, rice-paddy production increased by 4.07%, wheat 4.48%

Table 5. Simple annual growth rate of population and cereal production in South and South-East Asia

	Period		
	1961–73	1971–83	1981–93
Population	2.69	2.52	2.38
Cereal production	2.93	3.93	2.98

and other cereals 1.81%. Thus put together cereal production has remained high essentially because of rice and wheat. However, it is now clear that for this region the peak growth rate of rice was achieved between 1971–73 and 1981–83, but for wheat it was between 1961–63 and 1971–73. There has been a distinct decline in production growth rates in the decade of 80s. Therefore, some important questions are raised: (i) Would cereal production growth rate remain higher than population growth rate in the coming decades? (ii) While a decline in growth from the initial high is generally expected, would it remain around 3.0% in future also? (iii) What needs to be done to meet this objective?

Those questions can be analysed if not answered by considering sustainability of productivity and production at different levels. These are as follows: (i) Sustainability in large area such as South and South-East Asia, (ii) Sustainability in a country and within a country, (iii) Productivity potential through demonstrations, (iv) Sustainability on research farms – trends in improvement.

This analysis indicates that at the regional level, growth rates in the 80s in rice and 70s in wheat have not been maintained. There could be various reasons for this: (i) Initial high rates which are not sustainable because of limitations of biological system, (ii) Inputs have not matched the removal of nutrients, (iii) Socio-economic factors which effect growth.

The data on productivity of rice-paddy and wheat for the period between 1992 and 1994 clearly shows that there is almost no improvement in productivity in major producers of these cereals in South and South-East Asia (Table 6). Does it suggest that upper limit of productivity of these crops has been reached on a large area basis? Table 7 shows fertilizer consumption in some of the countries of Asia. Since this consumption does not pertain to rice, it is difficult to draw conclusions, but the experience shows that a large part of fertilizer is used for commercial crops, fruits and vegetable crops. Even so, it indicates a low level of consumption with a poor ratio of NPK. There is, of course, no mention of micronutrients. It is thus possible that stagnating yields are being caused by several factors, the imbalanced and low fertilizer use being an important one.

Table 6. Productivity (kg/ha) rice-paddy and wheat of major producers

	1992	1993	1994
Rice-paddy			
India	2618	2774	2817
Indonesia	4345	4375	4344
Bangladesh	2703	2678	2796
Vietnam	3334	3615	3462
Myanmar	2934	3055	2942
Philippines	2855	2955	3030
Wheat			
India	2394	2323	2420
Pakistan	1991	1947	1876
Bangladesh	1853	1846	1839

Rice–wheat rotation and rice–rice rotation

Rice–wheat rotation has emerged as an important cropping system in South and South-East Asia with a total area of 22.5 million ha, including China. The South Asian Countries – India, Pakistan, Bangladesh and Nepal together have 11.1 million ha where rice–wheat rotation is practised. The productivity of this system has been high and economically remunerative, therefore popular among farmers. However, there is a perception for the past few years that this system has become unsustainable. Consequently we need to ask the following questions: Is productivity of rice–wheat system declining, is it of rice or wheat? Is it true for the whole region or only of some parts? Is duration of rice crop a major factor for poor yield of wheat? Are there any soil, physical and biological factors which influence this rotation? Does this problem exist on research farms or in farmers fields? Are there any new pest problems – diseases, insects, nematodes, etc.? Is fertilizer and soil fertility management inadequate? Is water and water management a problem? Any other factor such as residue management and tillage? Will simulation models help?

Precise answers to these questions are difficult to find. A few years ago when work on this problem was initiated at Indian Agricultural Research Institute in 1992, three villages in Muradabad district of UP, about 160 km east of Delhi, were selected for the study. These three villages Nanhoowals, Hariana and Hamirpur have practised rice–wheat crop rotation for more than 20 years. These villages represent high, medium and low levels of productivity (Table 8). It appeared that the low-productivity village had a mild level of alkalinity and a low level of available phosphorus. Since the farmers are giving several irrigations to rice crop, it is likely that sodicity problems may arise in future. The physical properties of a 5-year and 15-year rice–wheat rotation systems at Karnal were also analysed. The hydraulic conductivity of the 15-year rotation was higher

Table 7. Fertilizer consumption of agricultural land in some countries of Asia (kg/ha)

	Period			
Country	N	P_2O_5	K_2O	Total
Bangladesh	71.5	14.5	6.1	92.1
India	48.5	14.7	5.0	68.2
Nepal	12.7	3.7	0.3	16.7
Pakistan	63.2	17.7	0.9	81.8
Philippines	38.2	9.2	5.9	53.3
Sri Lanka	49.0	16.4	24.4	89.8
Japan	117.1	142.1	95.4	354.6

FAO[19].

than 5-year rotation but is difficult to relate it to productivity. The biological activity, particularly microbial activity increased. The nature of microbes was not characterized. It is possible that the population of various kinds of microbes changes. Depending upon their chemical-producing ability these could be promotary or inhibitory to plant growth. This needs to be understood.

Changes in land use for crops in India

Significant changes in cropping pattern have occurred in India in the past four decades. In 1950–51, the coarse grains (maize, sorghum, pearl millet, minor millets, etc.) constituted 45% of the total cereal production coming from the area cultivated with cereals. Because of irrigation and other management factors a shift started among these crops by 1960. The maize crop experienced waterlogging in monsoon season in UP and Bihar and so did in Punjab. Consequently, rice which was not a crop of the region became a major cereal in monsoon season (Figures 2–4). Pearl millet or Bajra which occupied about 0.2 million hectare is almost non-existent in Punjab. As against this, rice is cultivated in 2 million hectare as opposed to its non-existence in 1960–61. Sorghum was a major cereal crop of Andhra Pradesh occupying more than 2.5 million hectare in 1970–71 but today it has come to 1.3 million ha. A major factor was the economics in farming. It has, however, resulted in changes which in the near future could lead to an adverse effect on production as well as human health. The changes in Punjab, an important grain-producing area, is described in some detail.

Table 8. Yield range of rice-paddy and wheat in the three villages of Muradabad District (UP)

Village	Rice-paddy (Q/ha)	Wheat (Q/ha)	Level of production
Nonhoowala	60–70	40–50	High
Hariana	35–50	35–40	Medium
Hamirpur	20–30	20–25	Low

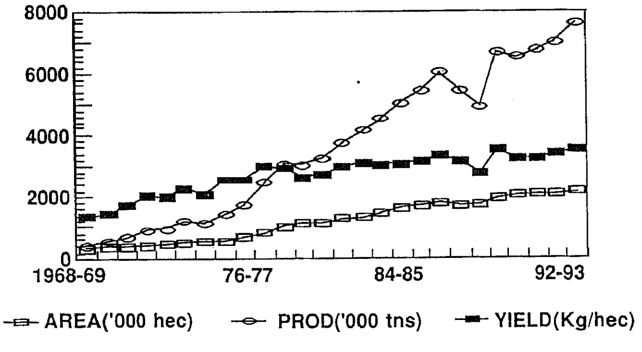


Figure 2. Change in land use pattern for rice in Punjab.

Prospects of rice–wheat production in Punjab

We identified Punjab, Haryana and UP as the key states for providing stability in production and for food security in the country[9]. In 1992, the Punjab contributed one-third of the production and two-thirds procurement of rice and wheat, among the key states. The sustainability of rice–wheat cropping system is a matter of concern for this region. Within Punjab, five districts, viz. Patiala, Jalandhar, Ludhiana, Sangrur and Amritsar had a growth rate ranging from 7 to 27% between 1971 and 1989. These districts contributed more than 60% rice and 50% wheat production. Since the market price was remunerative, the farmers were happy and so were bureaucrats and politicians, because this state became a model for agricultural development.

The consequences of high production of rice and wheat are no longer encouraging. There is stagnation or decline in area for wheat production (Figure 5) from 1984. The impact of increase in the area under rice on groundwater in five key districts of Punjab was analysed. It showed that area under rice increased at the rate of more than 10,000 ha annually since 1969 in all these districts, while the groundwater level in October (post-

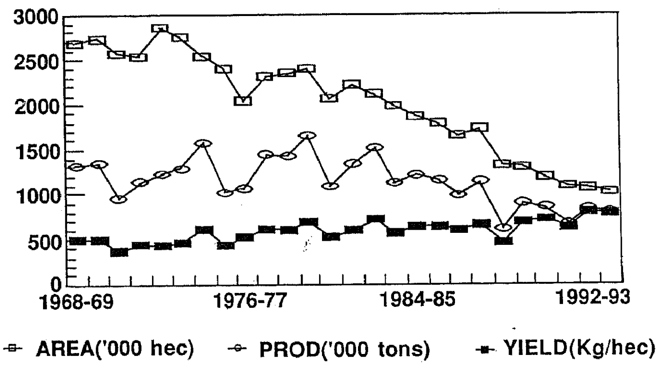


Figure 3. Change in land use pattern for sorghum in Andhra Pradesh.

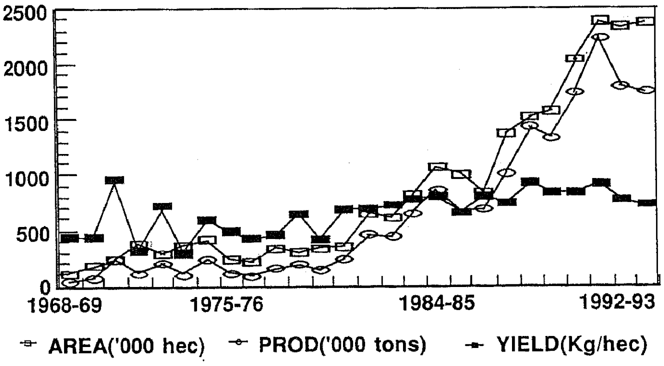


Figure 4. Change in land use pattern for rape seed and mustard in Rajasthan.

monsoon) has fallen at the rate of 0.17 to 0.46 in per annum (Figure 6). Thus in some of the districts the water level has gone down 3 m or more. This puts in a serious question on sustainability of rice production which is a major consumer of water. When there is success in an endeavour, the immediate gains make us forget the future of our coming generations. Farmers in Punjab and Haryana started planting rice in mid-summer, April and May to harvest two crops of rice. A high evaporative demand has brought the present situation which needs immediate attention.

Andhra Pradesh is an important state for production and contribution to food reserves in the country. Rice–rice rotation is predominant in the coastal area. A recent report[10] of the Andhra Pradesh Agricultural University on 'Stagnating Rice Productivity in Andhra Pradesh' brings out many factors including irrigation, diseases and pests, and soil problems as the causes of declining or stagnating yield.

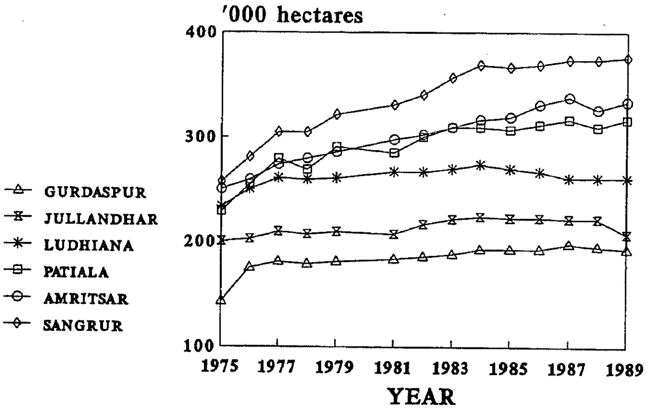


Figure 5. Expansion of wheat area in Punjab.

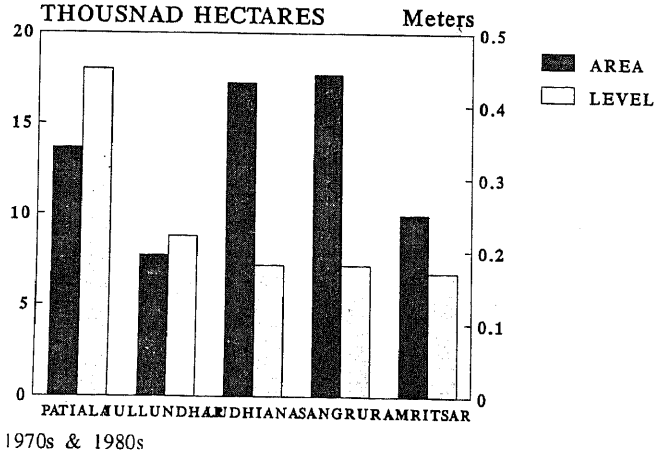


Figure 6. Groundwater fluctuations in October due to rice area expansion.

Groundwater pollution

Bajwa *et al.*[11] have investigated the problem of nitrate-nitrogen (NO_3–N) pollution of groundwater in Punjab where fertilizer use has been increasing. The samples were drawn from 236 tube-wells and more than 200 wells in cultivated area of several blocks. The NO_3–N content varied from 2.73 ± 1.22 to more than 10.0 mg NO_3–N per liter of water. The data on land use (Table 9) shows that the least amount of nitrate nitrogen occurred in rice–wheat system and the maximum was in vegetable crops. It was shown that the wheat crop which has a deeper root system is able to utilize nitrate from the lower levels. The vegetable crops received 15–20 t/ha of farm yard manure (FYM) in addition to 75 to 100 kg nitrogen per hectare. Therefore, the groundwater pollution with NO_3–N depends on the land use system and it is difficult to generalize the observation from any one cropping system.

Research for crop improvement

Crop improvement, particularly varietal improvement, has been a major objective of research in agriculture in South and South-East Asia. The collaboration between these countries and International Rice Research Institute, and CIMMYT from early 60s resulted in release of many high-yielding varieties. The IR8 in rice was the first to make impact on rice-paddy production. In India it gave rise to a variety, Jaya. However, there have been efforts to incorporate disease and pest resistance in new varieties, or to change their duration to fix them in a particular cropping pattern. In addition, there is a continuing effort to increase productivity, but on a large area basis or in experiments the yields of IR8 and Jaya have not been surpassed[12,13]. Data from IRRI is shown in Figure 7. Khush and his colleagues at IRRI are engaged in improving yield to 13.0 or 14.0 tons ha^{-1} of rice-paddy. There are indeed hybrids of rice which usually yield 0.5 to 1.0 tons ha^{-1} more than the existing varieties, but there is not much data to show if their sustainability has been evaluated. In the last five years the productivity of rice-paddy in China seems to be stagnating despite a larger area being planted with hybrid

Table 9. Nitrate concentration in groundwater samples under different land use systems

Land use systems	No. of tube-wells	% of wells containing 0–5 mg	% of wells containing 5–10 mg
Rice–wheat	68	97	3
Potato–wheat	34	94	6
Vegetables	42	83	17

Location of the remaining tube-wells could be classified under a well-defined land use system.

rice. Therefore, it is important that research in this region should pay more attention to causes of stagnation or decline in yield, and attempt to develop indices for deterioration of methods of cultivation, soil and environment. Have we to think of fixing an upper limit of production in a given region or soil to ensure sustainability?

The improvement of wheat at CIMMYT as well as in India also shows a slowing down as expected. But what is worrying is that the productivity in some districts is quite close to the best culture in coordinated trials. Again a greater emphasis on basic mechanisms related to environmental degradation including soils is required to ensure continued growth in production of rice and wheat which are the major cereals produced and consumed in South and South-East Asia.

Environment aspects of land cover change

There is a global concern for the global climate which according to IPCC[14] is already showing the signs of change because of increasing concentration of greenhouse gases due to human activity. Among the various greenhouse gases, carbon dioxide, methane and nitrous oxide have been associated with land cover change and agriculture. Deforestation, according to some, has contributed to increased CO_2 in the atmosphere. There are some models through which biomass of a forest cover could be estimated. These models need validation at the sites because it is not only rainfall but soil, composition of species in a forest, climate, etc. which determine the biomass of a forest. Similarly the estimates of contribution by soil organic carbon to atmospheric carbon dioxide need to be validated. As stated earlier, there is now evidence to suggest that forests are accumulating carbon in India rather than contributing it to the atmosphere.

The IPCC[15] report attributed 110 Tg methane production from rice-paddy, largely based on experiments in Europe and California, USA. These experiments did not represent the conditions in which rice-paddies grow in a large part of Asia[16]. Measurements in India, China, Australia and other countries have clearly shown far lower values than given by IPCC. However, the 1995 IPCC Report does not state methane emission from rice-paddies but includes it in anthropogenic emission without attributing a value for rice-paddies. It states that stabilization of CH_4 and N_2O concentrations at today's levels would involve reductions in anthropogenic emissions of 8% and more than 50% respectively.

Estimate of nitrous oxide shows its increasing concentration in the range of 3.0 to 4.5 Tg annually. Several sources have been identified, including the cultivated soils. How does agriculture influence production of nitrous oxide? If poor efficiency of nitrogenous fertilizers in tropical countries is one of the possibilities, then we have to obtain data. There are now techniques, such as the use of encapsulated urea with calcium carbide which reduces emissions of both methane and nitrous oxide (Figure 8).

Conclusions

1. Land cover and land use changes have occurred in South and South-East Asia as happened anywhere else in the last century and early part of this century. However, the present data indicates very little change in forest cover in the last two decades.
2. During the past three decades, food production, largely of rice and wheat increased at a rate higher than population growth rate. The region has become dependent on rice and wheat which have shown signs of stagnation in productivity.
3. In some high-productivity areas, there is evidence of changes in groundwater level, and also of groundwater pollution.
4. There has been very little progress, if any, in the productivity of rice as well as wheat since the

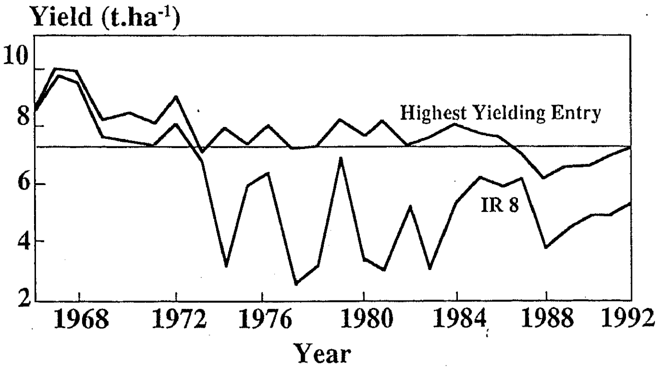


Figure 7. Grain yield of IR8 and the highest yielding single entry in dry seasons, 1966–90, at IRRI (G. S. Khush).

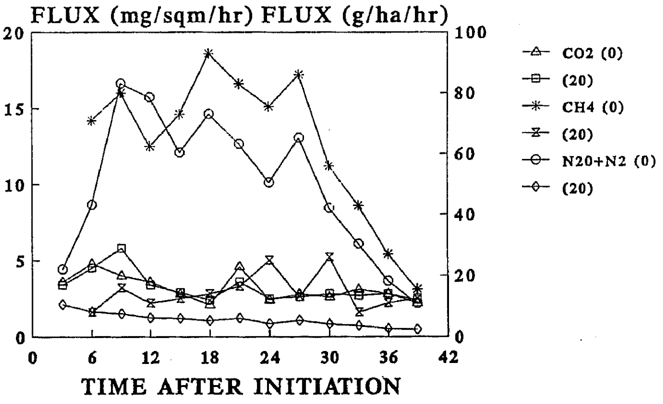


Figure 8. Effect of encapsulated calcium carbide on CO_2, CH_4 and N_2 flux in rice.

high-yielding varieties such as IR8, Jaya and kalyansona were released.

5. A decrease in production of coarse grain is a matter of concern. It is these crops in combination with tuber crops such as cassava and sweet potato which could have a profound effect on food security in combination with soybean and groundnut. Food technology should play an important role in future in making more effective use of these crops.
6. In India a greater emphasis on rainfed areas is essential to ensure sustainability of the present day high-productivity regions.

Action points

1. The problem of land use changes occur at microlevel that is the village level. Therefore, action should also start from there.
2. There is an urgent need to develop indices for unsustainability which could be used for monitoring different components of environment.
3. There is very little understanding of environmental economics in this region with an intensive involvement of various disciplines of science. Often arbitrary economic values are used which can lead to either complacency or alarm. Therefore, a new approach of working has to be developed.
4. A better database is needed to make use of models for aspects related to global change.
5. Regional programmes should be developed on land cover changes and agriculture sustainability which should include site visits, discussion with participants.

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Crystal structure of the peanut lectin – T-antigen complex. Carbohydrate specificity generated by water bridges

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Peanut lectin binds with high specificity to the tumour-associated disaccharide Galβ1–3GalNAc, generally known as T-antigen. The crystal structure of the complex of the lectin with the disaccharide has been determined at 2.5 Å resolution. Comparison of the structure with that of the corresponding complex with lactose reveals that the specificity of the lectin for T-antigen is generated primarily by two specific water-mediated interactions, probably the first instance where water-bridges have been demonstrated to be responsible for generating specificity in protein–carbohydrate interactions. The elucidation of the structure of peanut lectin–T-antigen complex also provides a framework for exploring peanut lectin-based prognosis and diagnosis of certain types of carcinoma.

THOMSEN-FRIEDENREICH antigen, generally known as T-antigen (Galβ1–3GalNAc), is a chemically well-defined tumour-associated antigen of non-oncofetal origin with a well-documented link to malignancy in man. This structure is generally expressed as O-linked glycans (Galβ1–3GalNAc–αSer/Thr), prominently so in more than 85% of human carcinomas such as those in colon, breast, bladder, buccal cavity and prostate, as well as on poorly differentiated cells[1-5]. It is, however, cryptic or absent in normal cells. Among the proteins that recognize T-antigen, peanut (*Arachis hypogaea*) agglutinin (PNA) is most widely used. Unlike other anti-T probes such as amranthin and jacalin, it does not bind to the more abundant cryptic T- and Tn-antigens (GalNAcα-O-Ser/Thr) which are the sialylated derivatives of the T- and Tn-antigens respectively[6-8]. It is for this exclusive specificity of PNA and its consequent usefulness as a diagnostic tool, that its importance has not diminished even after the advent of other T-antigen specific proteins including monoclonal antibodies[9]. The specificity of PNA is exploited widely for monitoring the differential expression of T-antigen for both the prognosis and the diagnosis of malignancies[10-13]. PNA has also found extensive use in the early detection of T-polyagglutinability which has proven to be life saving in numerous instances. Ability of PNA to differentiate between immature thymocytes and their mature counterparts has found applications in bone-marrow transplantations[14].

PNA is a 110 kDa homotetrameric non-glycosylated legume lectin with an unusual quaternary structure[15]. We report here the 2.5 Å crystal structure of the lectin complexed with T-antigen, refined to an R-value of 17.5%. The complex provides a detailed description of the geometrical features of PNA–T-antigen recognition and illustrates, perhaps for the first time, how water molecules could be largely responsible for carbohydrate specificity[16]. It also provides a framework for designing better tools for monitoring the expression of T-antigen.

Methods

Experimental

The protein was prepared by affinity chromatography on cross-linked arabinogalactan[17]. Crystals of the complex were grown from a hanging drop of 5 mg/ml protein in 0.05 M sodium phosphate buffer, pH 7.0, containing 0.2 M sodium chloride, 0.02% sodium azide, 1.5 mM Galβ1-3GalNAc and 12% (w/v) PEG 8000, equilibrated against 40% (w/v) PEG 8000 in the same buffer. A 2.5 Å data set was collected from a single crystal (space group $P2_12_12$, $a = 129.892$ Å, $b = 126.676$ Å, $c = 76.516$ Å) on a Siemens-Nicolet area detector mounted on a GX20 Marconi Avionics Rotating anode X-ray generator. Data were processed using XENGEN[18] (Table 1).

Refinement

The structure of the PNA–lactose complex refined to 2.25 Å resolution was used as the starting model for refinement after removing water and sugar molecules from it. Refinement was initially carried out using 3 Å resolution data employing PROLSQ[19] in the CCP4[20] suite of programs. Data in the higher shells were introduced step by step. Restraints for non-crystallographic symmetry were used in all but the final cycles. A difference map calculated after a few cycles of refinement showed clear density for the T-antigen in all the four monomers. All maps were inspected using

Table 1. Crystallographic data and refinement statistics

Data collection statistics	
Resolution	2.5 Å
Number of observations	65934
Number of unique reflections	33952
Data completeness	82%
R-merge*	0.128
Refinement statistics	
Resolution range	10–2.5 Å
Number of protein atoms	6976
Number of carbohydrate atoms	104
Number of solvent atoms	517
Other ions	4 Mg^{2+}, 4 Ca^{2+}
Total number of atoms	7605
R-cryst ($F > 2\sigma F$)	0.175
R-free	0.251
Root-mean-square deviation from ideal geometry	
Bond lengths	0.009 Å
Bond angles	1.7°

*R-merge $= \Sigma \mid I - \langle I \rangle / \Sigma \langle I \rangle \mid$.

FRODO[21]. In the absence of the crystal structure, the initial model of the T-antigen was constructed using the 'carbohydrate builder' routine in the 'biopolymer' module in the Biosym/MSI package, interfaced with InsightII. After fitting the T-antigen, water molecules were added in steps using 2Fo-Fc and Fo-Fc maps. Further refinement was carried out using XPLOR[22]. Engh and Huber parameters were used throughout the refinement. Omit type maps were used to inspect the model[23,24]. The model converged to a final R-value of 0.175 and the corresponding R-free was 0.251. The relevant refinement statistics are presented in Table 1.

Reliability and geometrical parameters

The stereochemistry of the model was checked using PROCHECK[25]. More than 90% of the residues are in the most favourable region in the Ramachandran map[26] (Figure 1) with no residue in the disallowed region. The Luzzati plot[27] calculated at the end of the refinement showed the coordinate error to be around 0.2 Å. The displacement parameters of the main chain atoms have the maximum, the minimum and the average values of 68.7, 2.0 and 10.9 Å^2 respectively. The corresponding values for the side chain atoms are 73.0, 2.0 and 12.9 Å^2. Those for the sugar atoms and solvent atoms are 36.8, 2.0 and 17.6 Å^2 and 91.3, 2.0 and 25.7 Å^2 respectively. Hydrogen bonds and interactions involving water molecules were identified using the criteria employed in the case of the PNA–lactose complex[28].

The coordinates and structure factors have been deposited in the Brookhaven Protein Data Bank, with accession codes, 1TEP and R1TEPSF respectively.

Results and discussion

General features

The four subunits in the tetrameric complex have the same structure. The root-mean-square (r.m.s.) deviations between the α-carbon positions when pairs of sub-units are superposed, range between 0.15 and 0.18 Å The range for all atoms is 0.23 to 0.25 Å while that for the atoms in the disaccharide is 0.15 to 0.24 Å. The tertiary and quaternary structure of the lectin in the T-antigen complex is the same as that in its complex with lactose[28]. The same is true about the location and the geometry of the carbohydrate binding site (Figure 2). Each subunit, 236 amino acids long, has the typical legume lectin fold and is made up of a six-stranded flat β sheet, a seven-stranded curved sheet, a small five-stranded sheet, which has a major role in connecting the other two large sheets, and a number of loops with differing length and conformation. The carbohydrate-binding region is generated by residues in four loops, 91 to 106, 125 to 135, 75 to 83 and 211 to 216, at one edge of the subunit.

PNA–disaccharide interactions

The electron-density for the bound T-antigen is well-defined in all the four subunits and it permits an unambiguous description of the PNA–T-antigen interactions (Figure 3 and Table 2). The four hydrogen bonds found in other legume lectin–galactose complexes occur in the present complex as well. They are Asp 83 OD1–galactose O3, Gly 104 N–galactose O3, Asn 127 ND2–galactose O3 and Asp 83 OD2–galactose O4 (Figure 3 *b*). The other invariant features of legume lectin–carbohydrate interactions, namely, the stacking of an aromatic residue, Tyr 125 in PNA, against the galactose ring and the proximity of Ala 82, are also observed in the complex[16]. These invariant interactions, involving the first three of the four loops mentioned earlier, define the primary common requirements for the binding of the galactose moiety to a legume lectin. The specificity of individual lectins to different sugars is determined mainly by interactions involving the fourth loop (211–216) (refs 29–31).

Comparison with the lactose complex and the structural basis of specificity

The structural basis for the specificity of PNA for T-antigen is best described in relation to the protein–sugar interactions in the lactose complex (Figures 3 and 4). Both the sugars are disaccharides, with galactose as the first residue. The second hexapyranose is β1-4 linked

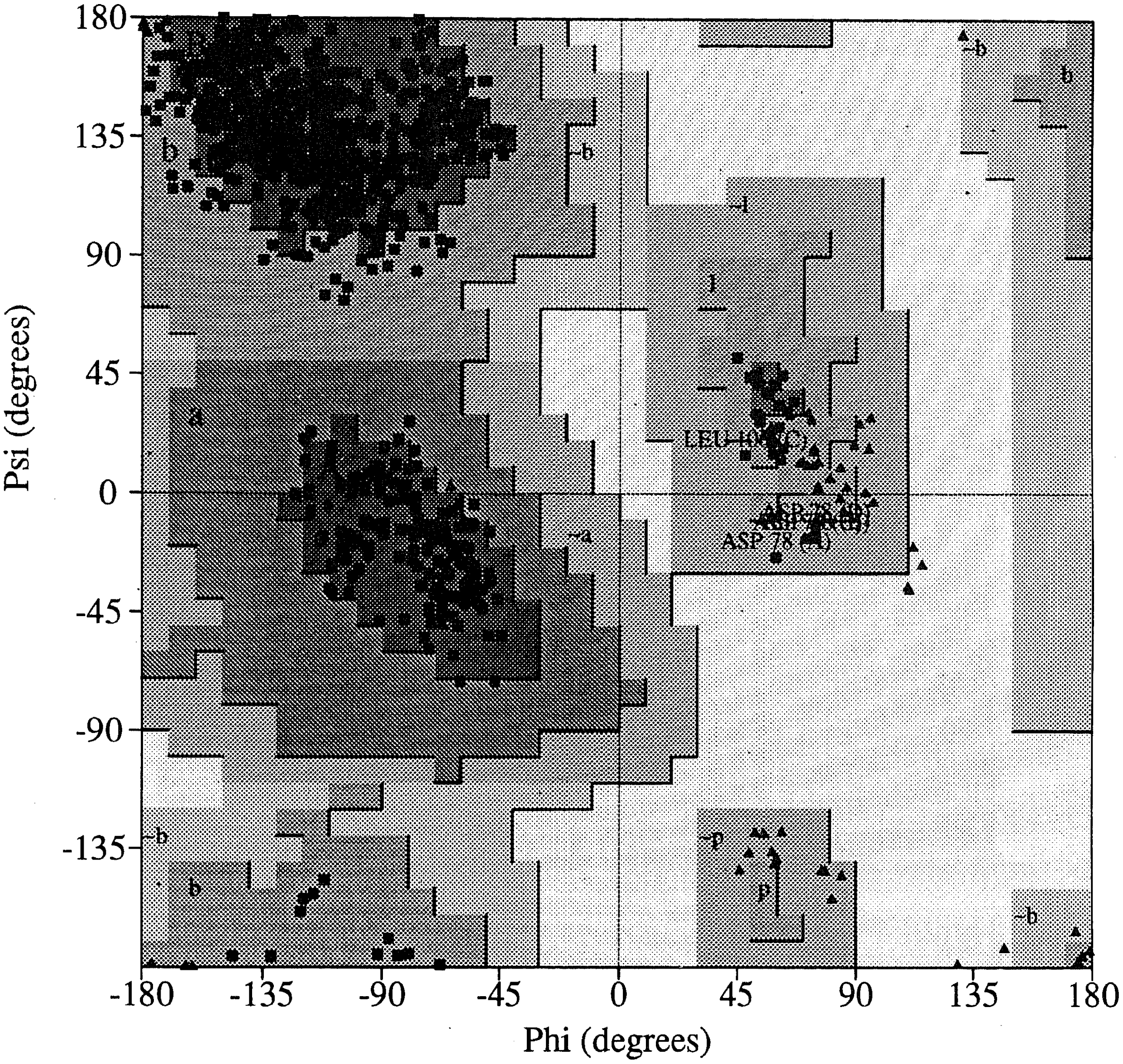

Figure 1. The Ramachandran map produced with PROCHECK. The squares and triangles represent non-glycine and glycine residues respectively.

glucose in the case of lactose while it is a β1-3 linked *N*-acetyl galactose in T-antigen. PNA, however, binds T-antigen 20 times more strongly than it binds lactose[4]. The invariant interactions, outlined earlier, exist in both the complexes. The other hydrogen bonds involving the galactose moiety (Ser 211 OG–galactose O4, Ser 211 OG–galactose O5, Asp 80 OD2–galactose O6) are also the same in the two complexes. In both the cases the galactose moiety is additionally linked to the protein through two water bridges (Gly 104 N–W2–galactose O2, Glu 129 OE2–W1–galactose O2). Thus the hydrogen bonded interactions involving galactose are the same in both the complexes. The same is true about the non-polar contacts. In the lactose complex, the second ring is connected to the lectin through two hydrogen bonds, both involving glucose O3, with Ser 211 OG and Gly 213 N. The differences in the linkage and the conformation of the two disaccharides are such that O4 of the second ring in the T-antigen occupies the same position as O3 of the second ring of lactose does, with respect to the lectin (Figure 4*a*). Therefore in the T-antigen complex, O4 of *N*-acetylgalactosamine is hydrogen bonded to Ser 211 OG and Gly 213 N. The number and the nature of non-polar protein–sugar contacts are also nearly the same in the two complexes. Therefore, the direct interactions of PNA with the two disaccharides are essentially the same and they do not explain substantially the higher affinity of the lectin for the T-antigen. The only additional interactions T-antigen has with PNA are two water bridges, involving the carbonyl oxygen of the acetamido group. One water molecule (W3) connects the oxygen atom to Ile 101 O, while the other water molecule (W4) connects it to Asn 41 ND2 and to Leu 212 N. These two water molecules exist in all the four subunits in the lactose complex as well. However, they interact only with the protein atoms in it, as there is no sugar atom in their immediate vicinity. Thus the high specificity of PNA for T-antigen and its 20-fold higher affinity for this saccharide than that for lactose, are generated essentially by these water bridges.

Comparison with other relevant lectin–carbohydrate complexes

The only other known structurally well-characterized

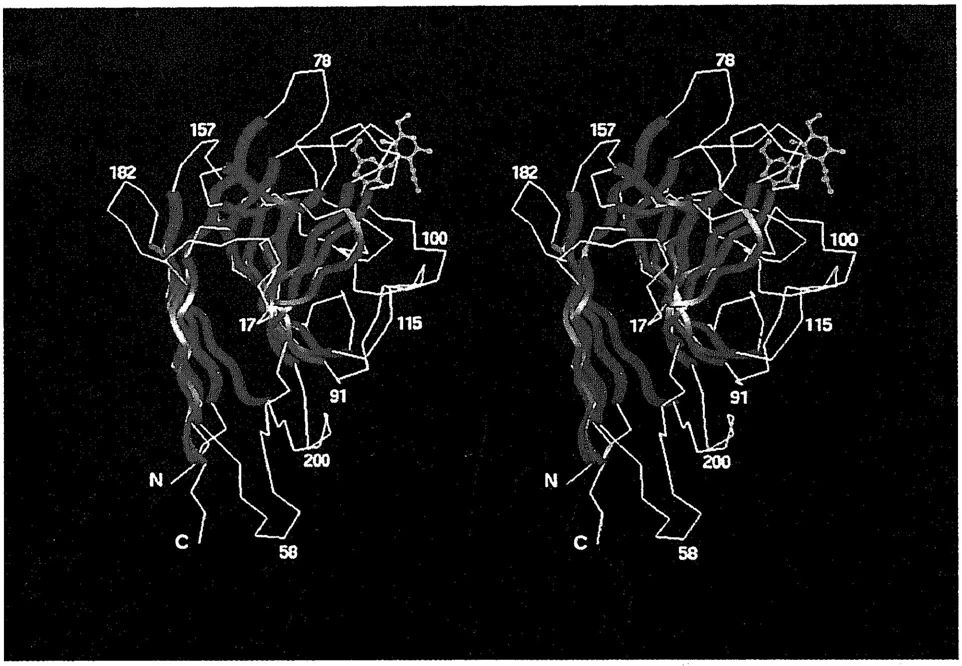

Figure 2 ***a*****.** Stereo view of a peanut lectin monomer with the bound T-antigenic disaccharide shown in cyan, ball and stick representation. The beta sheets and loops are coloured red and yellow respectively (Insight, Biosym/MSI).

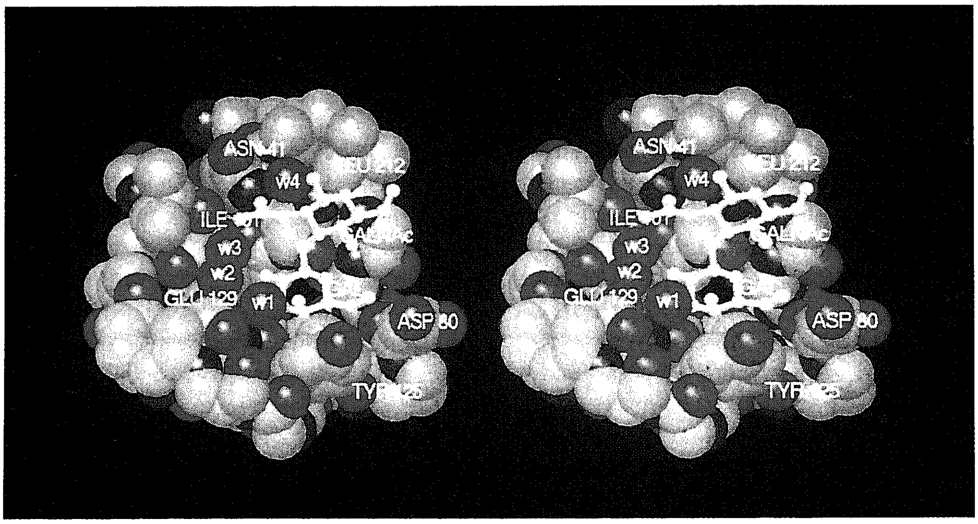

Figure 2 ***b*****.** Solid van der Waals surface (oxygen in red, nitrogen in blue and carbon in pink) of the carbohydrate-binding region with the disaccharide shown in ball and stick (Insight, Biosym/MSI).

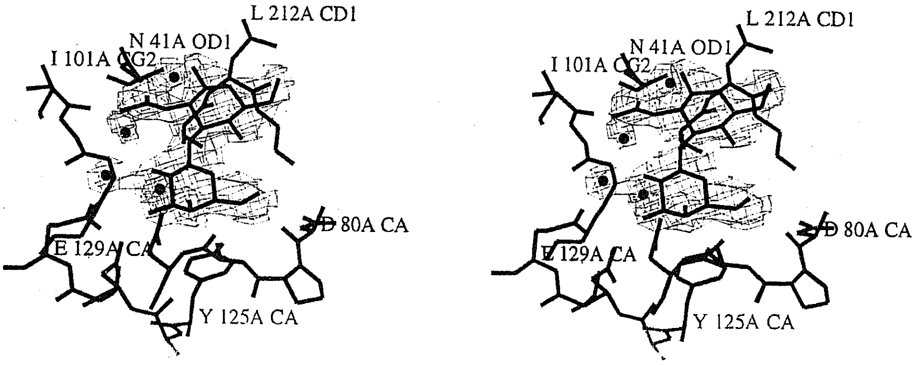

Figure 3 ***a***. Omit map corresponding to the sugar molecule and the water molecules involved in protein–carbohydrate water bridges. The surrounding peptide stretches are also shown. The map is contoured at 3.2σ level.

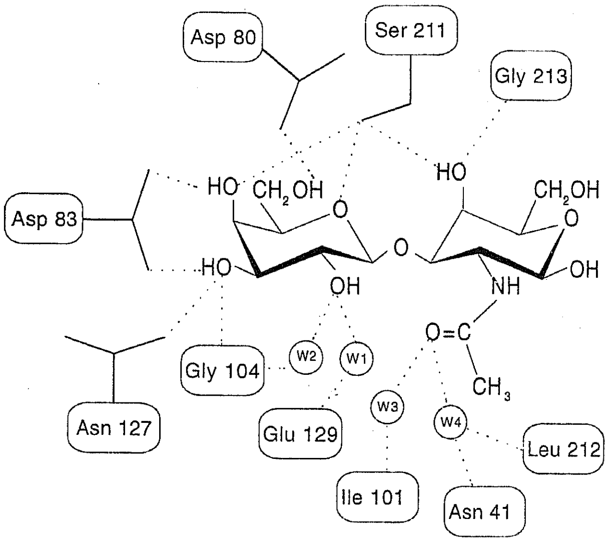

Figure 3 ***b***. Schematic representation of protein–carbohydrate interactions in the complex.

protein complex with the T-antigenic disaccharide is that involving the heat-labile enterotoxin (LT) from *E. coli*[32]. In this complex, however, only the galactose moiety interacts with the protein. The *N*-acetyl-galactosamine moiety has virtually no interaction with LT. This is not surprising as LT has evolved to recognize the terminal sugars (galactose and sialic acid) of the GM1 pentasaccharide, and not T-antigen. Thus, the complex of LT with the T-antigenic disaccharide does not reflect the protein's natural binding propensity unlike in the case of the PNA complex of the disaccharide.

The crystal structure of jacalin[33], the second lectin to be demonstrated to have T-antigen specificity, has been determined recently in our laboratory, in its complex with methyl-α-galactose. This tetrameric protein with a novel lectin fold has an unusual carbohydrate binding site in which the specificity is generated by a post-

Table 2. Protein–sugar interactions

Protein atom	Sugar atom	Distance (Å) in Mon1	Mon2	Mon3	Mon4
Hydrogen Bonds					
GalO3	Asp83 OD1	2.80	2.61	2.67	2.66
	Gly104 N	3.22	2.98	2.88	2.91
	Asn127 ND2	2.76	2.97	2.78	2.96
GalO4	Asp83 OD2	2.71	2.75	2.70	2.87
	Ser211 OG	2.97	3.18	2.81	2.54
GalO5	Ser211 OG	3.02	3.00	2.83	2.95
GalO6	Asp80 OD2	2.83	2.94	3.43	3.19
GalNAcO3	Ser211 OG	3.15	3.17	3.12	3.20
GalNAcO4	Ser211 OG	3.33	2.98	3.08	2.89
	Gly213 N	3.29	3.06	3.14	2.81

Water mediated interactions (Distances (Å) averaged over four subunits)

GalO2- -W1- -Glu129 OE1	(O2- -W1 = 2.94; W1- -OE1 = 3.36)
GalO2- -W2- -Gly104 N	(O2- -W2 = 2.95; W2- -N = 2.97)
GalNAcO7- -W3- -Ile101 O	(O7- -W3 = 3.04; W3- -O = 2.94)
GalNAcO7- -W4- -Leu212 N	(O7- -W4 = 2.81; W4- -N = 2.79)
- -Asn41 ND2	(W4- -ND2 = 2.61)

Residues less than 4 Å from any sugar atom

Asp80, Ala82, Asp83, Gly103, Gly104, Tyr125, Asn127, Ser211, Leu212 and Gly213

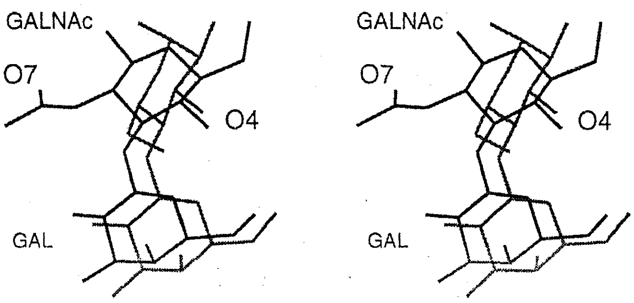

Figure 4 ***a***. Superposition of the T-antigenic disaccharide and lactose (light lines) as they occur in the respective complexes. The lactose has been shifted slightly for clarity as the galactose rings superpose almost exactly.

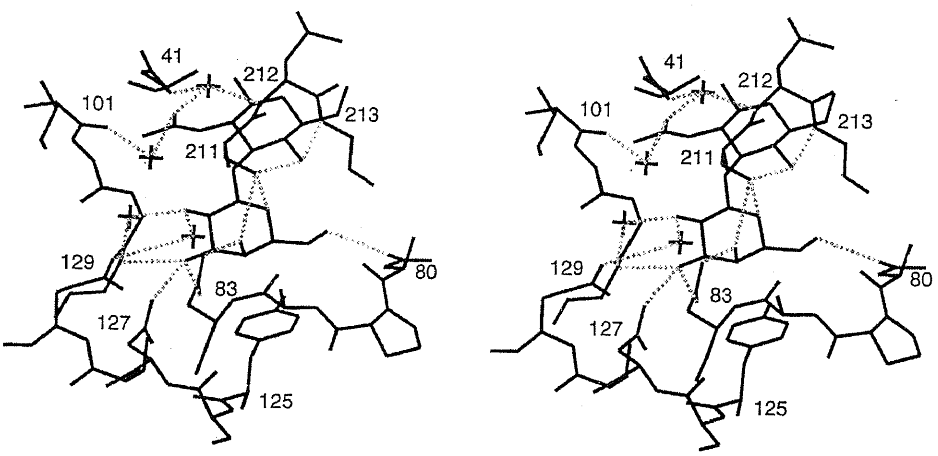

Figure 4 ***b***. Stereo view of the protein–T-antigen interactions in PNA. The crosses represent water molecules and the broken lines hydrogen bonds.

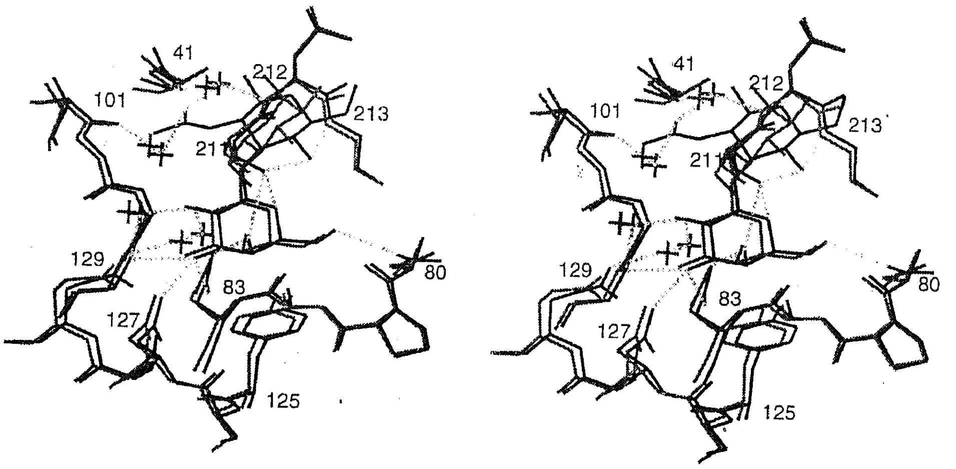

Figure 4 ***c***. Stereo view of the superposition of the protein–sugar interactions in the T-antigen (dark lines) and the lactose (light lines) complexes. Only hydrogen bonds in the T-antigen complex are indicated.

translational modification. The structure of its complex with T-antigen has not yet been determined. However the carbohydrate-binding site of jacalin bears no striking resemblance to that of PNA. Furthermore, the protein-galactose interactions in the two lectins are different in nature. Thus the structural determinants of T-antigen specificity in jacalin and PNA are entirely different.

Concluding remarks

Water in the hydration shell can be considered to be an extension of the protein surface and structural water can effectively mediate carbohydrate–protein interactions by modifying the contours of the binding site. In addition to providing a detailed characterization of the PNA–T-antigen interactions, the crystal structure of the complex provides probably the first instance of carbohydrate specificity generated by water bridges. In addition to its intrinsic importance in relation to the growing body of detailed knowledge on protein–carbohydrate interactions, the information provided by the structure could be invaluable for the design of a PNA-based carcinoma marker.

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Toward a solution for the Himalayan puzzle: Mechanism of inverted metamorphism constrained by the Siwalik sedimentary record

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Since the mid-19th century, inverted sequence ('hot-side-up') of metamorphic rocks in the Himalaya has formed a controversial subject, which has drawn an increasing attention in recent years. Models to explain this phenomenon are either 'thermal' (a heating event in the middle to upper parts of the crust directly responsible for the inverted metamorphism) or 'structural' (tectonic disruption of a normal metamorphic sequence). Data on the heavy-mineral analysis of the Siwalik sedimentary rocks derived from the Himalaya are consistent with 'structural' models. Metamorphic index minerals preserved in the Siwaliks record successive denudation of lower- to higher-grade metamorphic rocks of the Himalaya preserved in the Lower through Upper Siwalik sediments, suggesting differential denudation of metamorphic zones in the Himalaya over the past 18 million years. We argue that this pattern of denudation and deposition, in conjunction with other geological and theoretical considerations, constrains a plausible 'structural' model for the inverted metamorphism in the Himalaya, according to which the apparent inversion of metamorphic sequence was a product of differential uplift and denudation of metamorphic zones probably related to imbricate thrusting and internal ductile shearing of the metamorphic rocks in a collisional tectonic setting.

MOUNTAIN building (orogenesis) encompasses a complex set of changes in rocks both at depth (metamorphism, magmatism and structural deformation) and on the surface (uplift and denudation). The process of regional metamorphism in mountain belts such as the Himalaya requires heat and pressure, and as such, degree of metamorphism should normally increase from top to bottom in the Earth's crust (i.e. a 'hotside-down' sequence). One of the long-recognized and long-standing problems in Himalayan geology has been an actual explanation for the 'inverted' ('reversed' or 'hotside-up') metamorphism observed in various sections of the Himalayan orogen[1]. Although this controversial aspect of Himalayan geology has received much attention over the past two decades, the subject is more than a century old. Mapping of the Himalayan metamorphic terrain by geologists in the Calcutta-based Geological Survey of India during the second half of the 19th century demonstrated that the degree of metamorphism increases from the bottom upward the topographic (and structural) levels. Impressed by his observation of the inverted metamorphism in the Garhwal Himalaya, Richard Oldham[2] in 1883 remarked that 'this is but part of the great Himalayan puzzle'.

Understanding the cause(s) of this phenomenon is undoubtedly important for unraveling the metamorphic and tectonic evolution of the Himalaya. However, its significance is not limited to the Himalaya. Inverted metamorphism has been found in several other mountains; for example, in the North American Cordillera (California), the Scandinavian Caledonides, the European Alps and the Sanbagawa metamorphic belt of Japan. Although inverted metamorphism can be brought about by various processes in different orogens, studies of the Himalayan inverted metamorphism provide better clues to tackle these problems because mountain building in the Himalaya is quite young (and indeed still active) and thus various models can be separated and tested with better accuracy than in ancient orogens, and also because Himalayan geology enjoys a rich tradition of concepts and data pertaining to inverted metamorphism. It is thus surprising to hear from St-Onge[3] that 'Tilley (1925) in his classic account of the metamorphic zones of the Southern Highlands of Scotland, was probably the first to recognize the existence of an inverted metamorphic zonation'.

This paper offers a new approach to constraining the cause(s) of inverted metamorphism in the Himalaya. Rather than merely relying on studies of metamorphic rock assemblages and structures presently seen in the Himalayan mountains (which has been done by numerous authors in the past), this new approach utilizes the erosion record of the Himalayan metamorphic terrain as preserved in the foreland sediments (the Siwalik Group) to shed light on the models proposed for the Himalayan inverted metamorphism. At first, however, a brief review of various models proposed for the Himalayan inverted metamorphism is necessary for our discussion.

Models of inverted metamorphism

Figure 1 shows a geological map of the Himalayan orogen with the following divisions from north to south:

(i) The Trans-Himalayan and Kohistan-Ladakh Batholith (Cretaceous–Eocene); (ii) the Indus–Tsangpo Suture Zone, marking the initial plate boundary between the Indian and Asian tectonic plates along which the Tethys ocean closed; (iii), the Tethys Himalaya representing the Cambrian–Eocene marine sediments of the Tethys deposited on the northern margin of the Indian plate; (iv) the Higher Himalayan Crystalline Complex, composed of amphibolite-facies metamorphic rocks and Tertiary granites; (v) the Lesser Himalaya consisting of Precambrian–Paleozoic sediments and low-grade metasediments and various pre-Himalayan granites; and (vi) the Sub-Himalaya (or the Siwalik Hills) containing freshwater sediments of Cenozoic age deposited in the foreland basin of the Himalaya. These lithotectonic divisions are bounded by large scale faults (Figure 1). Of these, the most relevant structure to our discussion is the Main Central Thrust (MCT) which has brought the Higher Himalaya over the Lesser Himalaya, although its location in various parts of the Himalaya has been controversial[4,5].

The rocks of the Tethys Himalaya and the Sub-Himalaya are essentially unmetamorphosed. The phe-

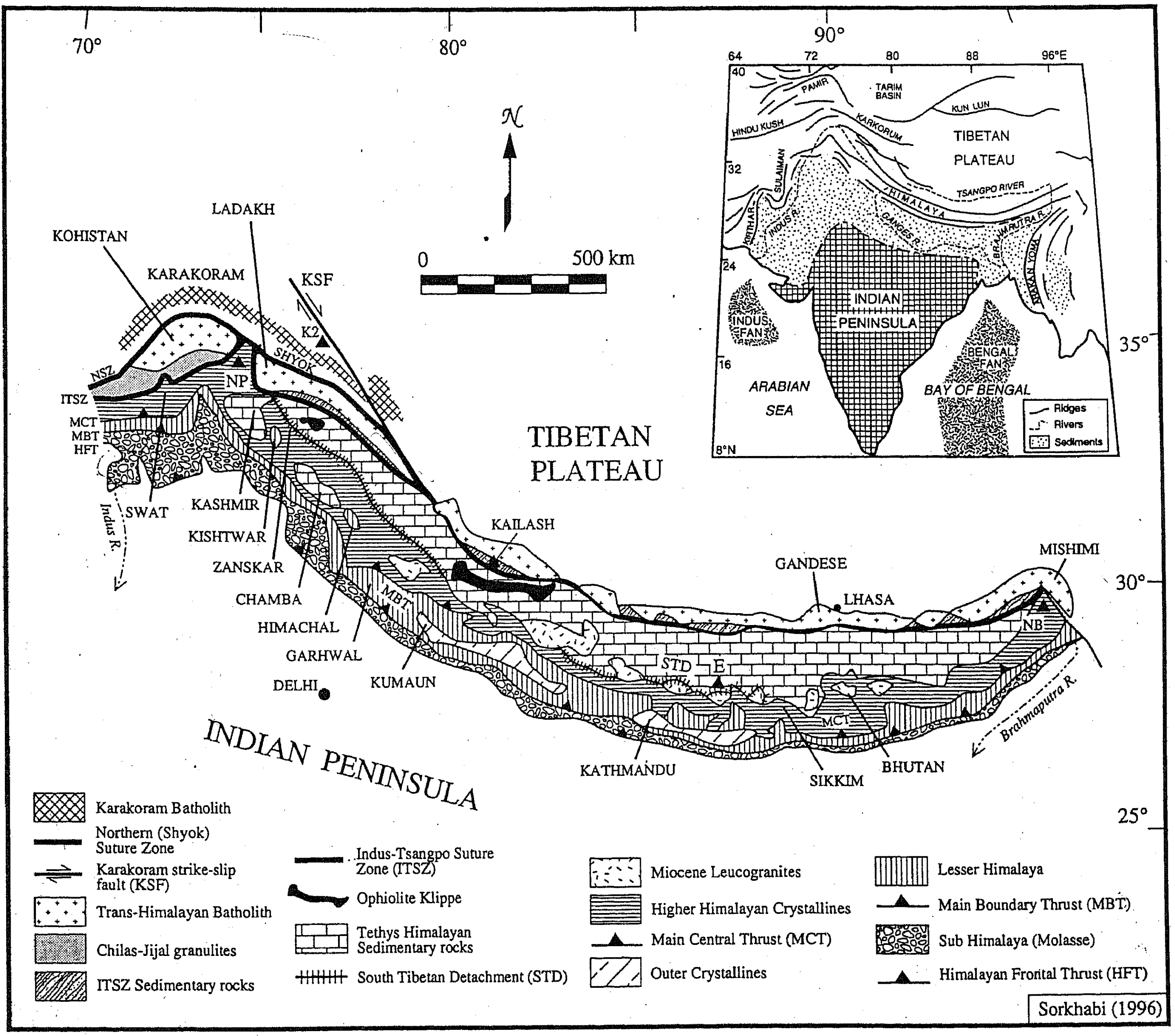

Figure 1. A geological map of the Himalaya, showing various lithotectonic divisions bounded by major faults. The map also shows the relationship between the Himalayan mountains and the depositional basins for sediments derived from the Himalaya.

Toward a solution for the Himalayan puzzle: Mechanism of inverted metamorphism constrained by the Siwalik sedimentary record

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Since the mid-19th century, inverted sequence ('hot-side-up') of metamorphic rocks in the Himalaya has formed a controversial subject, which has drawn an increasing attention in recent years. Models to explain this phenomenon are either 'thermal' (a heating event in the middle to upper parts of the crust directly responsible for the inverted metamorphism) or 'structural' (tectonic disruption of a normal metamorphic sequence). Data on the heavy-mineral analysis of the Siwalik sedimentary rocks derived from the Himalaya are consistent with 'structural' models. Metamorphic index minerals preserved in the Siwaliks record successive denudation of lower- to higher-grade metamorphic rocks of the Himalaya preserved in the Lower through Upper Siwalik sediments, suggesting differential denudation of metamorphic zones in the Himalaya over the past 18 million years. We argue that this pattern of denudation and deposition, in conjunction with other geological and theoretical considerations, constrains a plausible 'structural' model for the inverted metamorphism in the Himalaya, according to which the apparent inversion of metamorphic sequence was a product of differential uplift and denudation of metamorphic zones probably related to imbricate thrusting and internal ductile shearing of the metamorphic rocks in a collisional tectonic setting.

MOUNTAIN building (orogenesis) encompasses a complex set of changes in rocks both at depth (metamorphism, magmatism and structural deformation) and on the surface (uplift and denudation). The process of regional metamorphism in mountain belts such as the Himalaya requires heat and pressure, and as such, degree of metamorphism should normally increase from top to bottom in the Earth's crust (i.e. a 'hotside-down' sequence). One of the long-recognized and long-standing problems in Himalayan geology has been an actual explanation for the 'inverted' ('reversed' or 'hotside-up') metamorphism observed in various sections of the Himalayan orogen[1]. Although this controversial aspect of Himalayan geology has received much attention over the past two decades, the subject is more than a century old. Mapping of the Himalayan metamorphic terrain by geologists in the Calcutta-based Geological Survey of India during the second half of the 19th century demonstrated that the degree of metamorphism increases from the bottom upward the topographic (and structural) levels. Impressed by his observation of the inverted metamorphism in the Garhwal Himalaya, Richard Oldham[2] in 1883 remarked that 'this is but part of the great Himalayan puzzle'.

Understanding the cause(s) of this phenomenon is undoubtedly important for unraveling the metamorphic and tectonic evolution of the Himalaya. However, its significance is not limited to the Himalaya. Inverted metamorphism has been found in several other mountains; for example, in the North American Cordillera (California), the Scandinavian Caledonides, the European Alps and the Sanbagawa metamorphic belt of Japan. Although inverted metamorphism can be brought about by various processes in different orogens, studies of the Himalayan inverted metamorphism provide better clues to tackle these problems because mountain building in the Himalaya is quite young (and indeed still active) and thus various models can be separated and tested with better accuracy than in ancient orogens, and also because Himalayan geology enjoys a rich tradition of concepts and data pertaining to inverted metamorphism. It is thus surprising to hear from St-Onge[3] that 'Tilley (1925) in his classic account of the metamorphic zones of the Southern Highlands of Scotland, was probably the first to recognize the existence of an inverted metamorphic zonation'.

This paper offers a new approach to constraining the cause(s) of inverted metamorphism in the Himalaya. Rather than merely relying on studies of metamorphic rock assemblages and structures presently seen in the Himalayan mountains (which has been done by numerous authors in the past), this new approach utilizes the erosion record of the Himalayan metamorphic terrain as preserved in the foreland sediments (the Siwalik Group) to shed light on the models proposed for the Himalayan inverted metamorphism. At first, however, a brief review of various models proposed for the Himalayan inverted metamorphism is necessary for our discussion.

Models of inverted metamorphism

Figure 1 shows a geological map of the Himalayan orogen with the following divisions from north to south:

(i) The Trans-Himalayan and Kohistan-Ladakh Batholith (Cretaceous–Eocene); (ii) the Indus–Tsangpo Suture Zone, marking the initial plate boundary between the Indian and Asian tectonic plates along which the Tethys ocean closed; (iii), the Tethys Himalaya representing the Cambrian–Eocene marine sediments of the Tethys deposited on the northern margin of the Indian plate; (iv) the Higher Himalayan Crystalline Complex, composed of amphibolite-facies metamorphic rocks and Tertiary granites; (v) the Lesser Himalaya consisting of Precambrian–Paleozoic sediments and low-grade metasediments and various pre-Himalayan granites; and (vi) the Sub-Himalaya (or the Siwalik Hills) containing freshwater sediments of Cenozoic age deposited in the foreland basin of the Himalaya. These lithotectonic divisions are bounded by large scale faults (Figure 1). Of these, the most relevant structure to our discussion is the Main Central Thrust (MCT) which has brought the Higher Himalaya over the Lesser Himalaya, although its location in various parts of the Himalaya has been controversial[4,5].

The rocks of the Tethys Himalaya and the Sub-Himalaya are essentially unmetamorphosed. The phe-

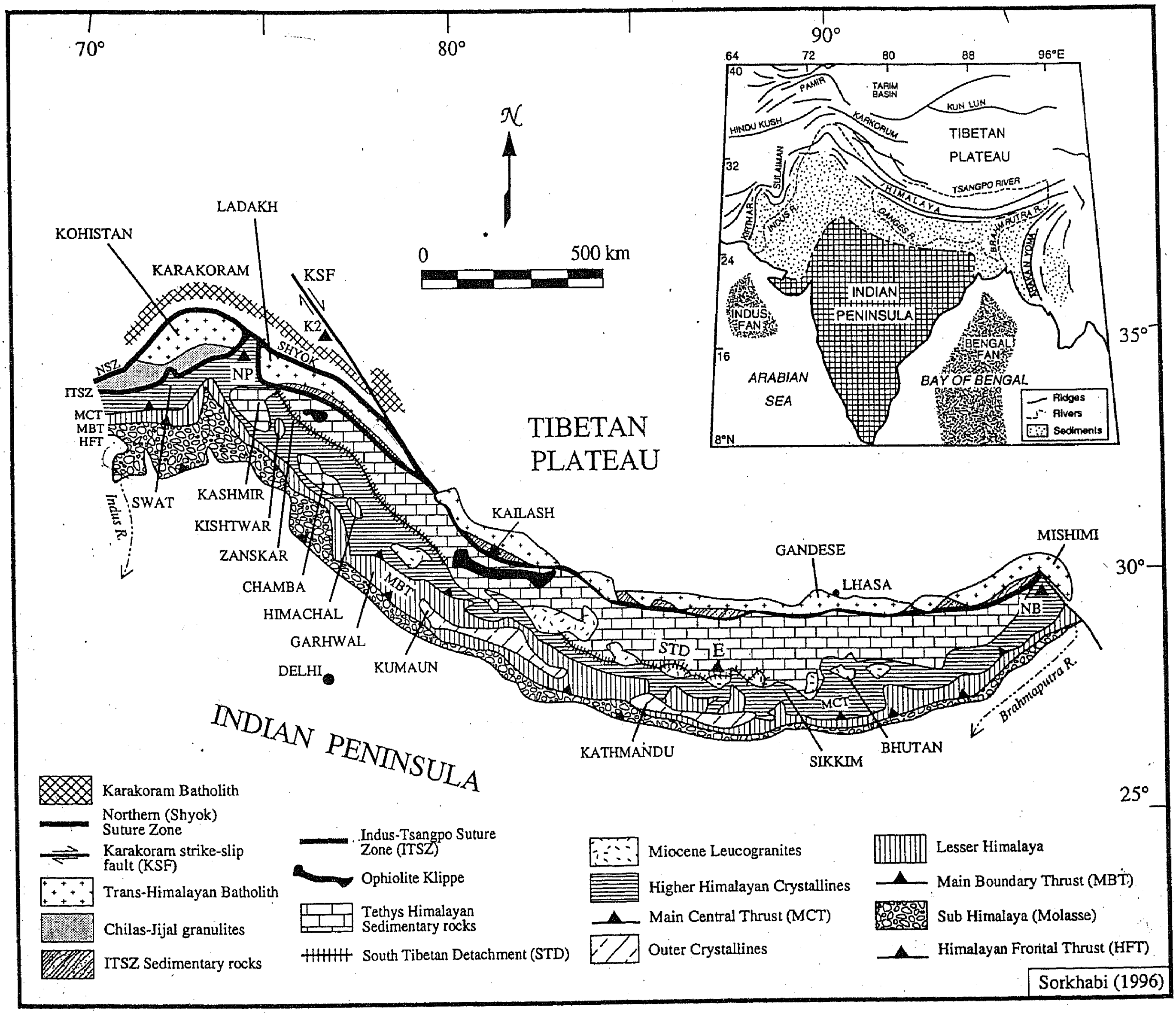


Figure 1. A geological map of the Himalaya, showing various lithotectonic divisions bounded by major faults. The map also shows the relationship between the Himalayan mountains and the depositional basins for sediments derived from the Himalaya.

nomenon of inverted metamorphism involves the Higher Himalaya, the MCT and the Lesser Himalaya. The regional metamorphism in the Himalaya range from the chlorite and biotite through garnet, staurolite, kyanite to sillimanite grade (from greenschist to upper amphibolite facies). Figure 2 depicts the distribution of metamorphic minerals in a section of the Himalaya[6].

Models proposed for the cause of Himalayan inverted metamorphism may be categorized into two broad groups[7,8] described below.

(i) 'Thermal Models', in which a heat source in the middle to upper parts of the crust was the direct cause of metamorphic inversion. As such, these models imply that the inverted metamorphism is an original (genetic) pattern in the thermal structure of the Himalayan rocks. Thermal Models include the following:
Model (1), Large-scale igneous intrusion in the Higher Himalaya[9–12];
Model (2), Overriding of a cold crust (the Lesser Himalaya) by a hot crust (the amphibolite-facies HHC) in an intracontinental subduction setting along the MCT that leads to the folding of paleo-isotherms and downward conductive heating of the Lesser Himalaya (the so-called 'hot-iron' model) (originally proposed by Le Fort[13]; revised in various forms by other authors[5,14–17]);
Model (3), Intense frictional heating on the MCT[6,14,18–20].
(ii) 'Structural Models', in which a post-metamorphic tectonic process inverted a normal (hotside-down) metamorphic sequence into a hotside-up sequence. Structural Models include the following (numbers beginning with 4, following the thermal models):
Model (4), Large-scale recumbent folding of metamorphic isograds (the folded crystalline nappe model) and tectonic transport of this nappe by the MCT (originally suggested by Löczy[21]; supported and elaborated on by others[22–27]);
Model (5), Tectonic juxtaposition of metamorphic assemblages of different ages or different lithostratigraphic units along major thrust faults[28–34]; or more clearly, post-metamorphic differential movement of individual metamorphic zones of a single prograde metamorphic phase as imbricate thrust sheets[35,36];
Model (6), Post-metamorphic ductile shearing of rocks on a small scale distributed across the Himalayan metamorphic zones (the germinal idea suggested as 'syn-metamorphic shear-folding of metamorphic zones' according to Frank and colleagues[25,26]; ductile thrusting and shear zone model of Brunel[37]; and a fully developed model by Jain and Manickavasagam[38]; see also references 8, 39–41).

Given the present status of knowledge, it is difficult

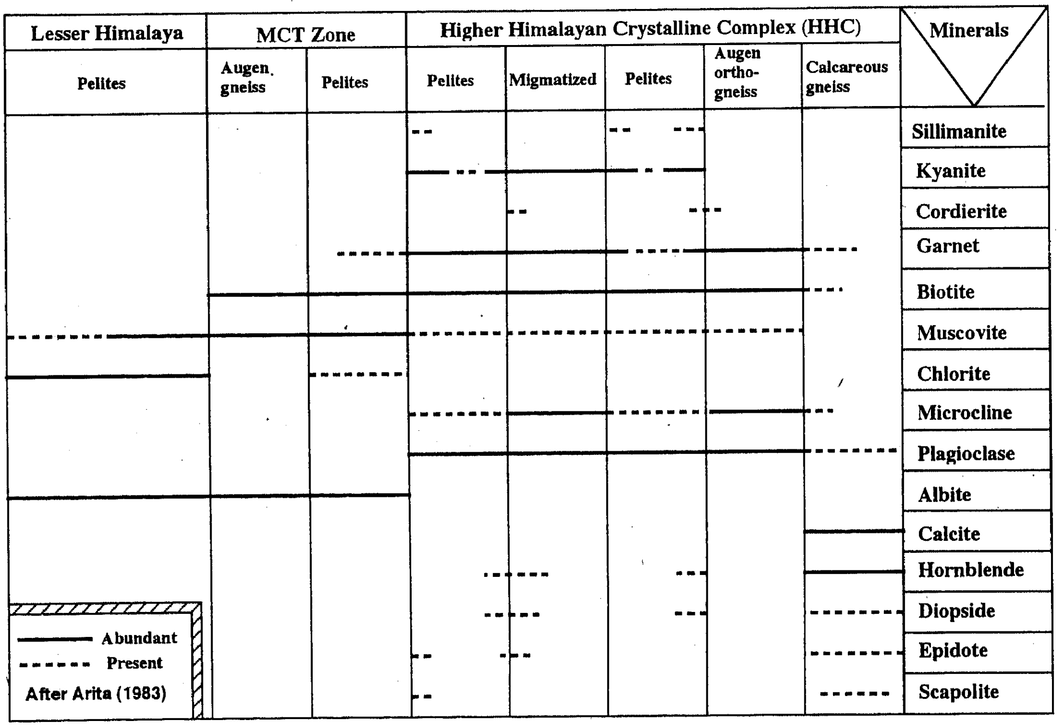


Figure 2. Distribution of various minerals in pelitic, psammitic and calcareous rocks of the Lesser Himalaya, the Main Central Thrust (MCT) zone and the Higher Himalayan crystalline complex. This figure is based on a petrographic study of rock samples across the Modhi Khola traverse in the Annapurna area of Nepal Himalaya. Modified after Arita[6]. Note that the grade of metamorphism increases from the Lesser Himalaya through the MCT zone to the core of the Higher Himalaya.

to determine the chronological boundaries between the Tertiary-age orogenic processes of metamorphism, plutonism, thrusting and folding in the Himalaya. Indeed, several authors referenced above have tried to combine some of these models and propose a multi-process model.

All of the models proposed for the Himalayan inverted metamorphism have been debated by numerous authors (some of whom were only referenced above due to lack of space), and the authors have provided structural, petrologic and thermobarometric data from the Himalaya to support or refute some of these models. However, none has been widely accepted as a unique solution for the whole Himalaya or even for a single sector of the Himalaya. In this paper, we adopt an entirely different approach, and synthesize the heavy-mineral analysis data from the Siwalik sediments to constrain these models.

The Siwalik molasse

The Siwalik Group exposed in the Sub-Himalayan zone is composed of clastic sediments (forming sandstone, shale, clay and conglomerate) transported by fluvial agents and deposited in basins developed in front of the rising Himalaya during Neogene times[1,42]. They are 'molasse' sediments in a truly Alpine sense, and numerous sedimentologic studies have established that the Siwaliks were derived from the Himalayan mountains[43–46]. Since the Siwaliks are rich in fossil content, their biostratigraphy is also well established in several sectors of the Himalaya, and over the past two decades magnetostratigraphic studies have tightened the deposition chronology of the Siwaliks. The Siwalik Group with a thickness of 5000–6000 m has been traditionally divided into Lower, Middle and Upper formations (Figure 3).

In the Potwar Plateau region of the Pakistan Himalaya, where the Siwaliks have been best studied, the Lower Siwaliks range in age from 18.3 Ma to 10.8 Ma and include the Kamliāl and Chinji stages; the Middle Siwaliks (10.8–5.1 Ma) include the Nagri and Dhok Pathan stages; and the Upper Siwaliks (5.1–0.7 Ma) consist of the Tatrot, Pinjor, and Boulder-conglomerate stages[47–50]. In the Arung Khola area of the central Nepal Himalaya, Tokuoka *et al.*[51] have adopted a different stratigraphic classification for the Siwaliks. According to them, the Arung Khola Formation consists of lower,

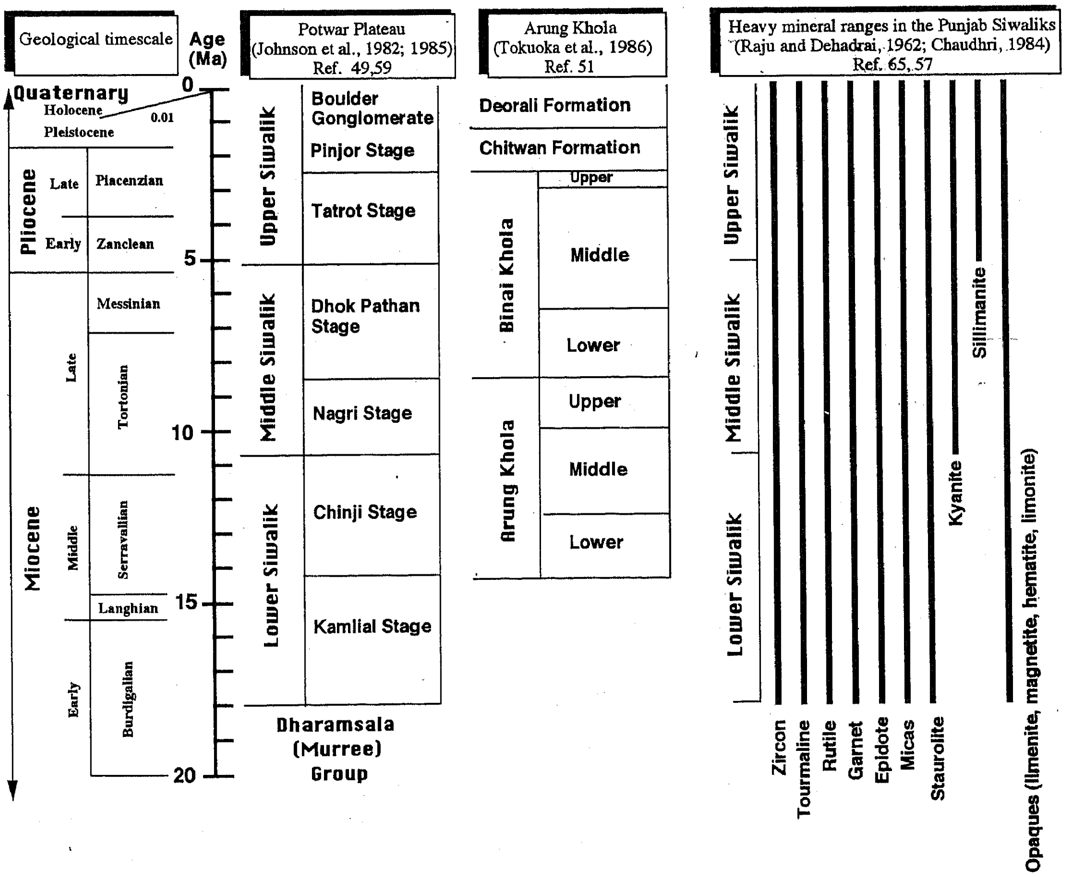


Figure 3. Stratigraphic classification of the Siwalik rocks in the western Himalaya and the Nepal Himalaya, and occurrence of heavy minerals in the Siwalik Group. References are given in the figure.

middle, and upper zones, corresponding in age to the Chinji and Nagri stages of the western Himalaya. The Binai Khola Formation beginning at 8.5 Ma also consists of lower, middle and upper zones, corresponding to the Dhok Patan and Tatrot stages. The Chitwan Formation (cobble conglomerates) beginning at 2.5 Ma is correlated with the Pinjor stage, and the Deorali Formation with the boulder-conglomerates of the western Himalaya (Figure 3).

Heavy-mineral analysis of the Siwalik Group

Heavy minerals in a sedimentary rock are those minerals heavier than quartz and feldspar, and practically defined as minerals with a specific gravity of over 2.9 g/cm^3 (which is the specific gravity of bromoform used to concentrate heavy minerals); they usually constitute less than 1 per cent of a sedimentary rock[52,53]. Metamorphic index minerals include chlorite, biotite, garnet, staurolite, kyanite and sillimanite, in an increasing order of metamorphic grade. These minerals are sequentially formed from the metamorphism of pelitic sediments (clays and shales) with an increase in temperature and pressure in the continental crust[54]. Metamorphic index minerals are also included in the assemblage of heavy minerals if the provenance of the heavy minerals were a metamorphic terrain. Of the metamorphic index minerals, the micas (chlorite, biotite and muscovite) occur in platy habit and have specific gravity in the range of 2.6–3.2 g/cm^3, and thus they cannot be completely separated by treatment with bromoform. For these reasons, the micas are often not considered in heavy-mineral analyses. Furthermore, biotite and muscovite are found in both igneous and metamorphic rocks. On the other hand, the presence of garnet, staurolite, kyanite and sillimanite in the heavy minerals of a sedimentary suite is diagnostic of a high-grade metamorphic provenance[53].

Heavy mineral analysis has been widely used for provenance (source area) studies, paleogeographic reconstructions and stratigraphic correlations. Reviews of this subject are given by Pettijohn[53], Blatt *et al.*[55] and Morton[56]. Over the past three decades, a number of heavy-mineral analyses of the Siwalik molasse have been carried out, mainly in the western Himalaya of India, and only a few studies from Nepal. A detailed review of these studies is out of the scope of this paper. For the sake of our discussion, we reference those papers, in which numerical values of the heavy minerals (usually expressed in percentage frequency) were documented. Furthermore, since we are concerned with metamorphic index minerals, only data for garnet, staurolite, kyanite and sillimanite are compiled here. (However, in some cases where data for micas were given, they have also been plotted, but the mica percentage frequencies should not be considered seriously for the reasons mentioned above.) In this paper, we do not consider the heavy-mineral analyses of the Murree (Dharamsala) Group of sediments, which underlie the Siwaliks, because very few data have been reported and because the stratigraphic (deposition) ages of these sediments are not precisely known.

Figure 4 *a–i* shows the percentage frequencies of garnet, staurolite, kyanite and sillimanite for the Lower, Middle and Upper Siwaliks from various areas of the Himalaya obtained by various authors as follows (arranged geographically from west to east): Chaudhri[57] for the Punjab Himalaya; Sharda and Verma[58] for the Jammu area (Punjab Himalaya); Sinha[59] and Shukla and Verma[60] for the Dehra Dun area (Garhwal); Gill[61] for the Garhwal Himalaya; Soman[62] for the Nainital area (Kumaun); Tandon[63] for the Ramnagar area (Kumaun); Chaudhri[57] for the Kumaun Himalaya; and Chaudhri and Gill[64] for the Nepal Himalaya.

Two major observations are noted from these plots (Figure 4): (1) Overall, the frequency percentages of metamorphic minerals increase through time from the Lower through Upper Siwaliks; (2) While garnet and staurolite occur in all Siwalik formations, kyanite appears in the Middle Siwalik sediments and continues its presence in the Upper Siwaliks, and sillimanite appears in the Upper Siwaliks.

These two observations seem to reflect a 'real distribution' of these minerals rather than 'subjective data' because they arise from studies of various parts of the Siwaliks and by various authors. The first point indicates that the Himalayan metamorphic terrain, which has supplied these detrital minerals, has experienced an accelerated uplift and erosion throughout the past 18 million years (Neogene times) during which the Siwalik sedimentation took place.

The second point is a confirmation of a finding originally made by Raju and Dehadrai[65] and published in this journal more than three decades ago (see Figure 3). These authors argued that staurolite seems to be a 'stratigraphic marker' for the Lower Siwaliks, kyanite for the Middle Siwaliks and sillimanite for the Upper Siwaliks. This observation was in contrast to an earlier study by Krynine[66], who reported that heavy minerals were 'generally equally well distributed throughout the whole Siwalik series'. However, Krynine[66] did not present any quantitative data for this claim. The observation of Raju and Dehadrai[65] was subsequently supported by Sinha[59], Tandon[63], Chaudhri[67] and several other authors mentioned above (see also Figure 4). The only author (other than Krynine[66]) who reported kyanite from the Lower Siwaliks was Soman[62]. However, in his data set, the majority of the samples from the Lower Siwaliks did not yield kyanite, and only two of them yielded 0.6 and 0.8 per cent kyanite (which is statistically insignificant; see discussion in Blatt *et al.*[55], p. 308).

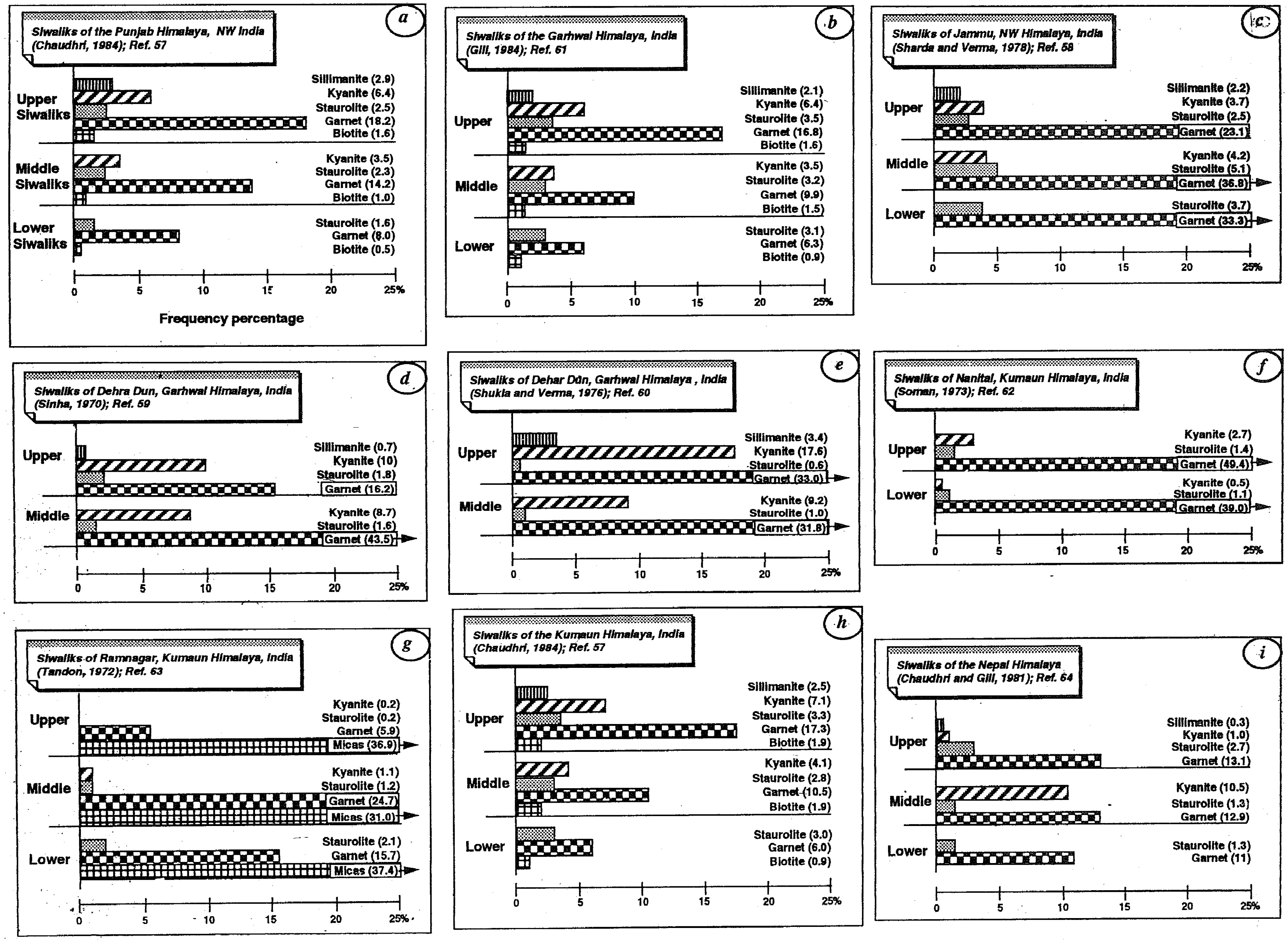


Figure 4. A compilation of heavy mineral analytical data from the Punjab, Garhwal and Kumaun Himalayas of India and the Nepal Himalaya, showing the frequency percentage for metamorphic index minerals (garnet, staurolite, kyanite and sillimanite) from the Lower, Middle and Upper Siwaliks. These plots are based on data reported by various authors, each referenced in *a–i*.

Therefore, his data do not refute observation (2), i.e. kyanite may be a stratigraphic marker for the Middle Siwaliks and sillimanite for the Upper Siwaliks.

Most of the heavy-mineral studies on the Siwaliks have been made in the Punjab, Garhwal and Kumaun Himalayas of India. One study was reported by Chaudhri and Gill[64] from the Nepal Himalaya. These authors used 31 samples for the Lower Siwaliks, 14 samples for the Middle Siwaliks and 10 samples for the Upper Siwaliks. Their observation that kyanite appears in the Middle Siwaliks is also supported by an independent study by Hisatomi[68], who noted that kyanite appears in the Binai Khola Formation and is absent in the underlying Arung Khola Formation (see Figure 3 for stratigraphic correlations of these formations with those of the western Himalaya).

Provenance or diagenesis

Before we conclude that the heavy mineral assemblage in the Siwaliks mainly represents the denudation of their provenance (i.e. the Himalaya), it is essential to establish that the physical and chemical effects such as weathering (in the soil profile), abrasion (during transportation) and diagenesis (after deposition) on the observed diversity of these minerals were insignificant. Here we discuss this subject rather in a greater detail for three reasons; because it has not been treated in a systematic manner in the Himalaya; it is necessary to address this problem both for this discussion and for other heavy-mineral studies of the Siwaliks in the future; and it has implications for long-standing debates over the influence of provenance and non-provenance factors in the assemblage of heavy minerals in sedimentary rocks.

Several charts of resistance or stability of heavy minerals against non-provenance effects have been proposed based on experimental or natural studies (Figure 5). Our discussion will be focused on the relevance of these charts to the heavy mineral assemblage in the Siwalik molasse.

One of the pioneering studies on the role of chemical weathering in the alteration of mineral content of bedrock was made by Goldich[69] using soil profile and bedrock samples of the Morton granite gneiss in the USA. His study was supplemented by that of Dryden and Dryden[70]. A chart of weathering resistance of heavy minerals in soil profile resulting from these studies is shown in Figure 5 *a*. Goldich[69] and Pettijohn[71] noted that there was an apparent relationship between the resistance of a given heavy mineral and its position in the Bowen reaction series (which is an arrangement of minerals according to their crystallization from a magma). In other words, a mineral (such as olivine) formed at highest temperatures in the most anhydrous magma is less stable under surface conditions than a mineral crystallized under lower temperatures in the more hydrous magma. However, this 'mineral stability series' does not imply that minerals less resistant to weathering cannot survive in the soil profile. The effect of weathering will also depends on climate and erosion rate[56]. Indeed, studies of Recent sediments in modern rivers such as the Nile[72], which were subjected to extreme weathering in the tropical source area, show abundant occurrence of less stable minerals such as hornblende and garnet. In the Himalaya, with thin soil and high erosion rates, the effect of weathering on heavy minerals must be very minimal. This is evident from the fact that garnet, a very less stable mineral (Figure 5 *a*), occurs abundantly not only in the Upper Siwalik rocks but also in the Lower Siwalik rocks. This in itself demonstrates rapid uplift and denudation of the Himalayan rocks during the Neogene.

Another possible factor altering a heavy mineral assemblage is abrasion during mechanical transportation of sediments. Thiel[73,74] has studied this effect experimentally and proposed a stability chart of minerals (Figure 5 *b*). However, in natural settings the abrasion effect seems to be insignificant. For example, studies of heavy minerals in the Nile shows no decrease in the diversity of heavy minerals with distance from the source area[72]. The occurrence of kyanite, which is easily destructible by abrasion according to Thiel's experimental chart (Figure 5 *b*), in the Upper as well as Middle Siwaliks indicates that the abrasion influence can be ruled out.

A third factor is the sorting of sediments in a depositional basin according to their density and diameter. This condition of settling in water is usually expressed by hydraulic equivalent size (i.e. the difference in size between a given heavy mineral and the size of a quartz sphere with the same settling velocity in water). Rittenhouse[75] carried out an excellent study of the Rio Grande River sands and found a good relationship between hydraulic size of heavy minerals and their densities (with a correlation coefficient of 0.91; see Figure 5 *c*). van Andel[76] studied grain-size distribution of some detrital minerals supplied by the Rhine River, and found that pyroxene, hornblende and epidote, which have approximately similar densities, were differentiated according to their sizes, i.e. coarse sediments were richer in pyroxene and finer sediments in epidote. The sorting effect (hydraulic size, density and diameter) does not seem to account for the gross assemblage of heavy minerals in the Siwalik molasse. For example, zircon and garnet, which have very high densities and hydraulic sizes (Figure 5 *c*) are found in the Lower, the Middle as well as the Upper Siwaliks, indicating their deposition at all levels of the Siwalik foreland basin. In fact, most of the heavy minerals analyses (Figure 4) show that the abundance of garnet increases from the Lower toward

Upper Siwaliks; this is in contrast to the density of garnet. Similarly, epidote (which tends to be associated with fine-grained sediments) is found in all formations of the Siwaliks (Figure 3), and its abundance does not decrease from the Lower through the Middle to the Upper Siwaliks (see data in references 57–64) while the Upper Siwaliks are the coarsest sediments.

Pettijohn[71] compiled the occurrence of heavy minerals in sedimentary rocks of various geological ages (from the Precambrian to the Present) as reported in numerous publications. From this tabulation, he worked out a 'mineral persistence' scale for heavy minerals (Figure 5 *d*). He found a correlation between his 'mineral persistence' chart and the 'mineral stability series' of Goldich[69], discussed above, and argued that the persistence of heavy minerals through geological time is an artifact of 'intrastratal solution' rather than reflecting their provenance. Although Pettijohn's 'persistence mineral series' has been often cited (partly because of its discussion in his well-known textbook, *Sedimentary Rocks*), he himself pointed out the limitations of his compilation of data[71], and as such, it should not be used as standard scale for interpreting all heavy-mineral assemblages in a given locality as merely product of intrastratal solutions. van Andel[76] has given a thoughtful criticism of Pettijohn's interpretation and has noted that his 'mineral persistence' scale is biased toward stable cratonic sediments for pre-Cretaceous times. In Pettijohn's compilation, heavy-mineral analyses of the Tertiary and Quaternary sediments mostly came from orogenic settings while pre-Cretaceous studies were on deposits of cratonic provenance in North America[76]. Even if we accept Pettijohn's mineral persistence chart and interpretation for heavy minerals on a global (not local) scale, its application to the Siwalik molasse is highly questionable because the persistence mineral chart (Figure 5 *d*) is for heavy mineral assemblages dating back to Precambrian times, while the Siwalik molasse is less than 20 million years old.

Pettijohn's argument for 'intrastratal solution' has been studied in the context of diagenesis (i.e. physical and chemical changes in sediments after their deposition but before metamorphism) by Morton[56]. He noted that two stages of diagenesis may affect heavy minerals. One stage is 'epidiagenesis', or changes brought about by surface waters moving downward. Based on an experi-

Inceasing order

a Ref. 69, 70	*b* Ref. 73,74	*c* Ref. 75	*d* Ref. 71	*e* Ref. 56	*f* Ref. 56
Resistence to Weathering in soil profile (Goldich, 1938; Dryden, 1946)	**Abrasion resistence of minerals (Thiel, 1940, 1945)**	**Sorting effects on heavy minerals: Hydraulic size (and density) (Rittenhouse, 1943)**	**Persistence of heavy mineals in geologic time (Pettijohn, 1941)**	**Stability of heavy minerals during epidiagenesis* (Morton, 1985)**	**Stability during anadiageneis* (Morton, 1985)**
Olivine Augite Garnet Staurolite Hornblende Kyanite Biotite Monazite Tourmaline Zircon	Barite Siderite Fluorite Goethite Enstatite Kyanite Bronzite Hematite Augite Apatite Spodumene Hypersthene Diallage Rutile Hornblende Zircon Epidote Garnet Titanite Staurolite Microcline Tourmaline Quartz	Tourmaline 0.2 (3.1) Hornblende 0.2 (3.2) Diopside (Brown) 0.3 (3.4) Pyroxene 0.3 (3.4) Kyanite 0.3 (3.6) Apatite 0.4 (3.2) Diopside (Colorless) 0.4 (3.3) Hypersthene 0.4 (3.4) Titanite 0.5 (3.5) Baryte 0.5 (4.5) Garnet 0.6 (3.8) Zircon 0.9 (4.6) Ilmenite 1.0 (4.7) Magnetite 1.0 (5.2)	Olivine Actinolite Diopside Hypersthene Sillimanite Augite Zoisite Titanite Topaz Andalusite Hornblende Epidote Kyanite Monazite Staurolite Magnetite Illmenite Apatite Biotite Garnet Tourmaline Zircon Rutile	Olivine/ Pyroxene/ Amphibole. Titanite. Apatite. Epidote/ Garnet. Chloritoid/ Spinel. Staurolite. Kyanite. Andalusite/ Sillimanite/ Tourmaline. Rutile/ Zircon.	Olivine/ Pyroxene. Andalusite/ Sillimanite. Amphibole. Epidote. Titanite. Kyanite. Staurolite. Garnet. Apatite/ Chloritoid/ Spinel. Rutile/ Tourmaline/ Zircon.
		Correlation coefficient between hydraulic size and density: 0.91		***Epidiagenesis: changes due to surface water**	***Anadiagenesis: changes due to deep burial**

Inceasing order

Figure 5. Charts for stability or resistance of heavy minerals against weathering in soil profile (*a*), abrasion during mechanical transportation (*b*), hydraulic size and density (*c*), persistence through geological times (*d*), intrastratal solution or diagenesis (*e* and *f*). The least stable minerals occur at the top of the lists. The compilation is based on various sources referenced in the figure for each chart.

ment of flushing low pH groundwater, Morton[56] listed a chart of stability for heavy minerals during epidiagenesis (Figure 5 *e*). Although this experimental chart is not applicable to all sedimentary basins (due to variation in the chemistry and pH of surface waters), it is cited here to note that the epidiagenesis seems to have little effect on the Siwaliks. For example, according to Morton's chart (Figure 5 *e*), sillimanite is more stable than kyanite, which is more stable than staurolite. But this is the reverse sequence of what is found in the Siwalik heavy-mineral assemblage (see Figure 4). Also, the least stable minerals such as epidote and garnet (Figure 5 *e*) occur in all horizons of the Siwalik sediments (Figure 3).

Another stage of diagenesis which may affect the diversity of heavy minerals is 'anadiagenesis' or changes due to porefluids (intrastratal solution) during deep burial of sediments[56]. There are very few reliable data to quantify this effect, mainly because it is extremely difficult to separate this effect from the provenance factor. Nevertheless, Morton[56] believes that the Paleocene sediments of the North Sea derived from the Scottish Highlands provide a unique opportunity to study the effect of anadiagenesis, and from this study, he has proposed a stability chart for minerals (Figure 5 *f*). Even taking this chart at face value, it seems that anadiagenesis was not a significant factor for diversity of the Siwalik heavy minerals. Epidote, which is less stable than kyanite according to this chart (Figure 5 *f*), occurs in the Lower, Middle, and Upper Siwaliks (Figure 3). Had kyanite deposited in an appreciable amount in the Lower Siwaliks, the population of kyanite could have survived the effect of anadiagenesis.

Three reasons argue against any significant influence of non-provenance factors in the gross diversity and relative abundance of the Siwalik heavy minerals. First, the Siwalik molasse is geologically very young; second, uplift and erosion rates in the Himalaya have been rapid; and third, both stable (e.g., tourmaline) and unstable (e.g., epidote) minerals are found together in the Lower, Middle and Upper Siwalik rocks.

A final testimony comes from Pettijohn himself; he writes: 'If heavy minerals are newly derived from crystalline rocks, they are little worn. Cleaved fragments and more or less euhedral crystals characterize the assemblage. If, however, the 'heavies' are derived from earlier sediments, the less stable species tend to be absent and the more stable survivors show notable rounding' (Pettijohn[53], p. 206). Almost all heavy-mineral studies of the Siwaliks referenced above demonstrate that euhedral heavy minerals such as zircon indicating single-cycled sedimentation from the Himalayan crystalline terrain occur abundantly in all of the Siwalik formations irrespective of their stratigraphic (depositional) ages.

We thus support the conclusions of Sinha[59], Chaudhri[57,67] and Nanda and Tandon[77] that the heavy mineral assemblage of the Siwaliks directly reflects the erosion history of its provenance (the Himalaya).

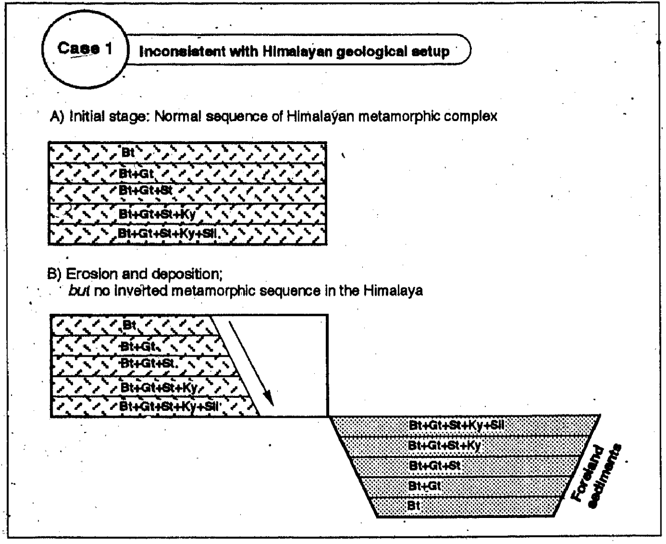
Toward a solution for the Himalayan puzzle

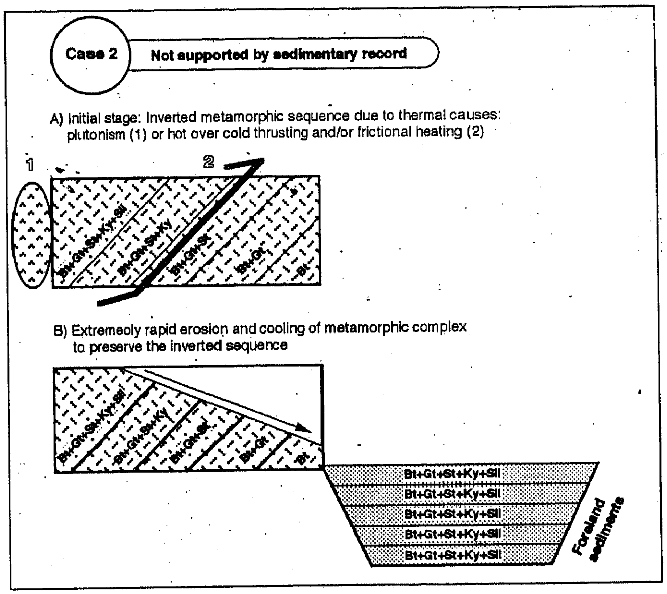
Sedimentary evidence from the Siwalik molasse presented in the preceding sections indicates that sillimanite (an index mineral of highest-grade regional metamorphism) appears in the Upper Siwalik, kyanite appears in the Middle Siwalik and continues its presence through the Upper Siwaliks, while staurolite, garnet, and micas (indicative of lower- to medium-grade metamorphic rocks) occur in the Lower through Upper Siwaliks. In other words, the higher-grade metamorphic zones in the Himalaya were subjected to denudation and deposition at a later time than the lower-grade metamorphic zones as noted by Sinha[59], Chaudhri[57,67] and Nanda and Tandon[77]. However, this pattern of denudation and deposition is not strange in itself; it may indicate successive denudation of a hotside-down metamorphic pile as shown in Figure 6, case 1. Possibly a normal metamorphic pile with the highest-grade rocks in the deepest levels was subjected to uplift and erosion though time, and therefore, the deepest rocks (in the sillimanite grade) were exposed at a more recent time than the lower-grade rocks originally occupying the upper levels of the metamorphic pile. However, what makes the erosion and sedimentary record of the Siwaliks so significant is that in the Himalaya the metamorphic pile is not in a normal sequence; it is hotside-up. For this reason, the sedimentary evidence from the heavy-mineral analysis of the Siwaliks puts important constraints on the models proposed for the inverted metamorphism in the Himalaya as described below.

Geochronological studies in the Higher Himalaya of India and Nepal show that the peak metamorphic conditions for the main metamorphic event that affected the rocks presently outcropped in the Higher Himalaya reached in the Late Oligocene–Early Miocene. ^{40}Ar/^{39}Ar ages determined on hornblende kinematically grown in the rock fabric on the hanging wall of the MCT are 20–25 Ma (refs 78–81). Since these are post-metamorphic cooling ages (recording temperatures of 500–550°C) and the peak metamorphic temperatures were on the order of 650–750°C (see Hodges *et al.*[82] for a review), the regional metamorphism must have occurred shortly before these cooling ages. Another age constraint is that the majority of the Higher Himalayan leucogranites probably formed during the main Himalayan metamorphism have been dated as 25–22 Ma by the U–Pb techniques[81,83,84]. Therefore, it is widely agreed that the main Himalayan metamorphism of rocks outcropped in the mountain was a Late Oligocene–Early Miocene event. The Siwalik sediments covering the past 18 million years are also widely considered to have been derived from the Himalaya.

If the inverted metamorphism were due to thermal causes in the middle to upper parts of the Himalayan crust, such as igneous intrusion (Model 1), shear heating along the MCT (Model 2) or hot-over-cold thrusting in an intracontinental subduction setting (Model 3), and if an already inverted metamorphic sequence were subjected to rapid uplift and erosion, one would expect to find both high-grade and low-grade metamorphic index minerals in the Lower Siwaliks through the Upper Siwaliks (Figure 6, case 2). Indeed, a condition necessary for the thermal models is that the inverted metamorphic pile should have cooled (uplifted and eroded) quite rapidly in order to preserve (quench in) the inverted metamorphic zonation[85,86]. But the sedimentary evidence is not consistent with this prediction of the thermal models. Model 1 and Model 2 are also refutable on the basis that the metamorphic effects of granitic intrusion and the MCT frictional heating would have been local, not on a Himalayan scale.

The structural models, on the other hand, predict that the inverted metamorphic sequence is a post-metamorphic event caused by tectonic disruption of metamorphic zones. This is consistent with the sedimentary record because the heavy-mineral analyses demonstrate that there were time gaps between deposition of lower- to higher-grade rocks; the garnet and staurolite zones were exposed to erosion and were deposited during Lower Siwalik times (18–11 Ma), the kyanite zone was exposed


Case 1
Inconsistent with Himalayan geological setup
A) Initial stage: Normal sequence of Himalayan metamorphic complex
Bt
Bt+Gt
Bt+Gt+St
Bt+Gt+St+Ky
Bt+Gt+St+Ky+Sil
B) Erosion and deposition;
but no inverted metamorphic sequence in the Himalaya
Bt
Bt+Gt
Bt+Gt+St
Bt+Gt+St+Ky
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky
Bt+Gt+St
Bt+Gt
Bt
Foreland sediments


Case 2
Not supported by sedimentary record
A) Initial stage: Inverted metamorphic sequence due to thermal causes: plutonism (1) or hot over cold thrusting and/or frictional heating (2)
1
2
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky
Bt+Gt+St
Bt+Gt
Bt
B) Extremeoly rapid erosion and cooling of metamorphic complex to preserve the inverted sequence
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky
Bt+Gt+St
Bt+Gt
Bt
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky+Sil
Foreland sediments

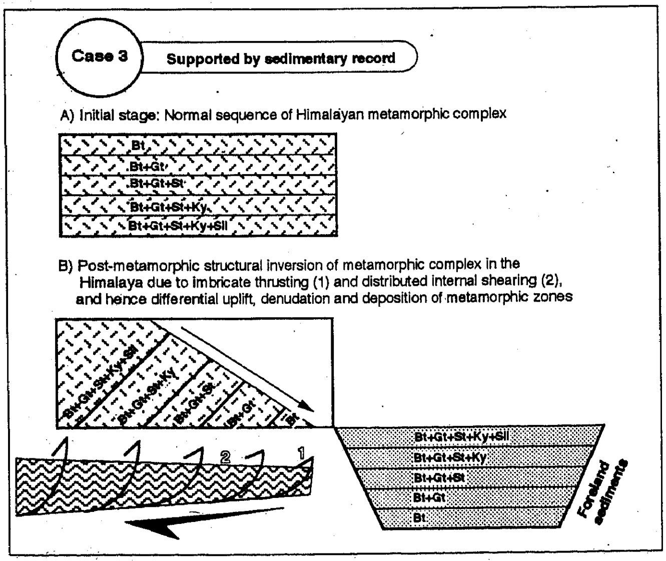

Case 3
Supported by sedimentary record
A) Initial stage: Normal sequence of Himalayan metamorphic complex
Bt
Bt+Gt
Bt+Gt+St
Bt+Gt+St+Ky
Bt+Gt+St+Ky+Sil
B) Post-metamorphic structural inversion of metamorphic complex in the Himalaya due to imbricate thrusting (1) and distributed internal shearing (2), and hence differential uplift, denudation and deposition of metamorphic zones
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky
Bt+Gt+St
Bt+Gt
Bt
2
1
Bt+Gt+St+Ky+Sil
Bt+Gt+St+Ky
Bt+Gt+St
Bt+Gt
Bt
Foreland sediments

Figure 6. Conceptual models to show relationships between a regional metamorphic sequence in a mountain range and its erosion and depositional record in a sedimentary basin. In case 1, sedimentation record is an inverse of metamorphic sequence; the highest-grade rocks were eroded and deposited at a later time, however, the metamorphic sequence in the mountain is normal (hotside-down). This is not applicable to the Himalaya, which exhibits an inverted metamorphic sequence. In case 2, a thermal event such as igneous intrusion, hot-over-cold thrusting or thrust-related frictional heating caused metamorphic inversion in the middle to upper parts of the crust, and subsequently the already inverted metamorphic pile underwent very rapid uplift and erosion. This is possible but it would need to yield higher-grade index minerals throughout the sedimentary record; there should not be long time gaps between inverted metamorphism and uplift-denudation-cooling (otherwise, the inverted metamorphic sequence could not have been preserved in the mountain). This is not consistent with the Siwalik sedimentary record. In case 3, a normal hotside-down metamorphic pile is transformed into an inverted (hotside-up) sequence by structural mechanisms (imbricate thrusting and distributed ductile shearing) after peak metamorphic conditions. This case predicts an apparent inverted metamorphic sequence in the mountain and time-gaps between deposition of lower- to higher-grade metamorphic zones (hence the sequential appearance of metamorphic index minerals in the sedimentary record). This scenario seems to be most consistent with the Himalayan metamorphism and the Siwalik sedimentary record. However, it should be noted that case 3 depicted in this figure is an ideal model. See text for discussion.

during Middle Siwalik times (11–5 Ma), and finally the sillimanite zone during Upper Siwaliks times (see Figure 3 for geological ages). Unfortunately, the available data from the heavy-mineral analyses do not specify more precise stratigraphic (depositional) ages of the Siwalik samples (i.e. what stage of the Lower, Middle or Upper Siwaliks), and therefore, a more quantitative understanding of the time-gaps between metamorphism and deposition of the rocks is not possible in this study. From the Siwaliks of Nepal, Hisatomi[68] reported the first occurrence of kyanite in lower zone of the Binai Khola Formation at 8.5 Ma (Figure 3).

A simple explanation for the observed pattern of heavy minerals in the Siwalik record is differential uplift, denudation and deposition of metamorphic rock assemblage in a manner that the highest-grade (deeper, hotter) rocks were uplifted and eroded at a later time than the lower-grade metamorphic rocks. This sequence of events is predicted by the structural models for the Himalayan inverted metamorphism.

Structural disruption (and hence differential uplift and erosion) of the metamorphic zone could have been accomplished by either recumbent folding (Model 4), multiple-thrusting (Model 5) or distributed ductile shearing (Model 6). Of these, Model 4 is less likely because recumbent folding of the rocks on the huge scale of the Himalaya is not observed; recumbent folds have been mapped as local features in the Himalaya (for example, the Donara nappe in Zanskar[27]). Recumbent folding requires stratigraphic inversion of rocks in the lower limb of the orogen-scale folded nappe; this is yet to be demonstrated in the Himalaya. Even in the classical study area of the Sikkim Himalaya, where Löczy[21] mapped Himalayan nappes, the nappes seem to be thrust nappes, not a recumbent fold. In the same area, the metamorphic zones are also separated by thrust faults as mapped by Sinha-Roy[5]. Therefore, we tend to support a combination of Models 5 and 6 as most likely causes for the inverted metamorphic sequence in the Himalaya. Both imbricate thrusting and distributed ductile shearing have been reported from the HHC and the MCT zone (see references for Models 5 and 6). And the sedimentary evidence discussed in this paper is consistent with the prediction of these models, i.e. multiple thrusting and internal ductile shearing of the metamorphic pile in the Higher Himalaya causing differential uplift and denudation of metamorphic zones and hence resulting in an inverted metamorphic sequence, which was not an original thermal pattern of the crust but a post-metamorphic structural disruption of metamorphic zones yielding now an apparent inverted metamorphism (Figure 6, case 3).

It should be noted that Figure 6 is an ideal picture showing fundamental concepts relevant to our discussion. The available data do not permit us to show the exact temporal pathways of the Himalaya rocks from crustal depths to mountain heights and then to depositional basins. For example, uplift and erosion rates may have varied through time. Moreover, the thrusting of the Lesser Himalaya over the Siwalik molasse along the Main Boundary Thrust has probably covered portions of the sedimentary record older than the Lower Siwaliks beneath the Lesser Himalaya and of which we have no information. Therefore, Figure 6, case 3 should be taken as a simplified model to interpret the Himalayan inverted metamorphic sequence in the light of the available data from the Siwalik sedimentary record and the structural setup of the Himalayan mountains.

A corollary of this study is that the foreland sediments in the Himalaya have preserved very important information about the thermal–tectonic evolution and uplift-denudation history of the Himalaya. Therefore, more sophisticated and better documented research is encouraged along these lines of thought.

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Shock-induced secondary vortices on a delta wing – An Euler simulation

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Earlier Euler simulations of transonic flow over delta wings, in spite of using very fine grids, have not shown the shock-induced secondary vortices. It is shown here that this results from an inherent deficiency of the computational grids employed. By employing a grid that correctly takes into account the length scales of the flow, for the first time, a lee-side flow, containing embedded cross-flow shocks and shock-induced secondary vortices, is obtained, right from the apex of the wing.

TRANSONIC flow over delta wings is of interest to highly maneuverable fighter aircraft and missiles. On a sharp-edged delta wing the flow separates from the wing leading-edges forming the primary vortices. As the incidence is increased the outboard flow beneath the primary vortex can attain supersonic speeds. This flow, which is highly spanwise, must turn to align itself with the wind-side flow at the leading-edge. Under appropriate conditions, this may lead to the formation of a cross-flow shock, and a shock-induced secondary separation, leading to the formation of a secondary vortex.

Euler simulations of transonic vortex on sharp-edged delta wings have been carried out by several investigators. Hoeijmakers *et al.*[1] have presented results for a 65 deg wing employing O–O grid with over 1.2 million cells. Bannink and Houtman[2] have employed a 196,000 cells C–O grid, and a 270,000 cells H–O grid has been used by Hitzel[3] in their computations. Longo[4] has presented Euler results on a 70 deg delta wing for a range of incidence, employing a H–H grid consisting of 460,000 points. While some of these calculations have partially captured the cross-flow shock, the shock-induced secondary separation has not been obtained in any of the earlier computations.

The conventional grids cannot resolve the flow near the wing-apex due to dissimilar length scales of the flow and the grid[5]. As the apex is approached, the flow in the transverse plane scales with the local semi-span, whereas the transverse length scales of a conventional grid, for example H–O, C–O, O–O or H–H grid, do not. This inherent feature of conventional grids cannot be overcome by a simple grid refinement.

As will be shown here, the poorly-resolved near-apex flow, computed using a conventional grid, cannot capture the cross-flow shock, and hence the shock-induced secondary vortex. The vortex resolution improves as one moves away from the apex; but the poor resolution of the near-apex flow delays the shock formation. A fully resolved near-apex flow appears critical for the simulation of the shock-induced secondary vortex. This has been achieved here by employing an embedded conical (EC) grid[5] which correctly takes into account the length scales of the flow.

Experimental investigations[6,7] on 65 deg sharp-edged delta wings at M_∞ (free-stream Mach number) of 0.85 show that the secondary separation is shock-induced for α (angle of attack) of 15 deg and above. We present below the computed flow over a 65 deg sharp-edged delta wing of zero thickness at M_∞ of 0.85 and α of 15 deg. An EC grid having 294,212 cells (a 68 (chordwise) × 32 (wing-normal) × 64 (spanwise) inner conical grid with a 76 × 32 × 64 outer H–O grid), for the half-wing is employed. A comparable conventional H–O grid having 311,296 cells (76 × 64 × 64) is also employed. The Euler method and the grid generation method are described in ref. 5.

Figure 1 shows the EC solution C_p contours in the first transverse cell plane (TCP) downstream of the

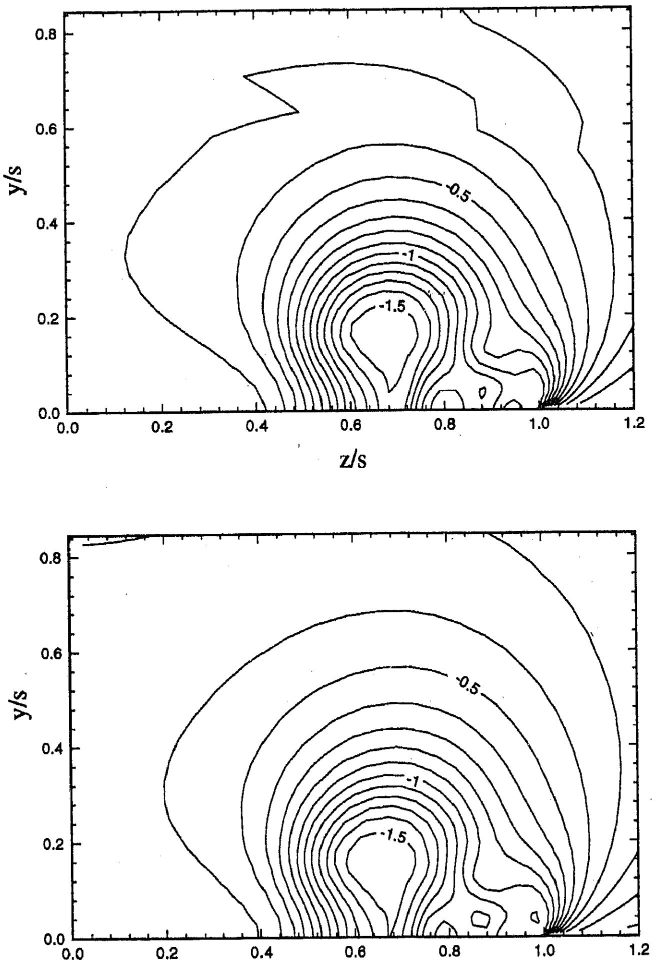

Figure 1. Contours of C_p at $x = 0.00128$ (1st TCP), and at $x = 0.3048$ (19th TCP).

apex, which is at $x = 0.00128$ (x, y, and z are the coordinates along the root-chord, wing-normal and spanwise directions, with the origin located at the wing-apex, and linear dimensions are scaled by the root-chord), and at $x = 0.3048$ (19th TCP). The vortex is seen to be fully resolved right from the first TCP. Further, the similarity of the plots in the two TCPs indicates the nearly conical nature of the flow in the fore part of wing. A cross-flow shock, located on the lee-surface at about $z/s = 0.72$ (s is the local semi-span), can be seen from the contours. The secondary vortex core can also be seen from the C_p contours.

A shock wave can occur only when M_b, the Mach number component normal to local isobaric surface, exceeds one[8]. Figure 2 *a* shows contours of M_b in the first TCP, with the C_p contours superimposed over it. We see that just ahead of the cross-flow shock, the value of M_b is more than one. Figure 2 *b* shows the conically projected velocity vectors in the first TCP, along with a few streamlines through this vector field. Downstream of the cross-flow shock, the near surface streamlines moves away from the surface, leading to the formation of the secondary vortex. We thus see that the present EC grid computation is able to capture the experimentally observed shock-induced secondary vortex, right from the apex.

In comparison, Figure 3 shows the H–O solution C_p contours at $x = 0.02158$ (5th TCP) and at $x = 0.3048$ (19th TCP). The vortex structure is seen to be poorly formed even in the 5th TCP, which is due to fewer grid points in the wing-normal direction to resolve the flow. The poorly formed vortex generates a lower suction in the vortex core and on the lee-surface. Due to the poorly-formed pressure and velocity fields, M_b does not exceed one in the 5th TCP, and hence a cross-flow shock cannot occur near the apex. However, as one moves away from the apex, the vortex becomes better

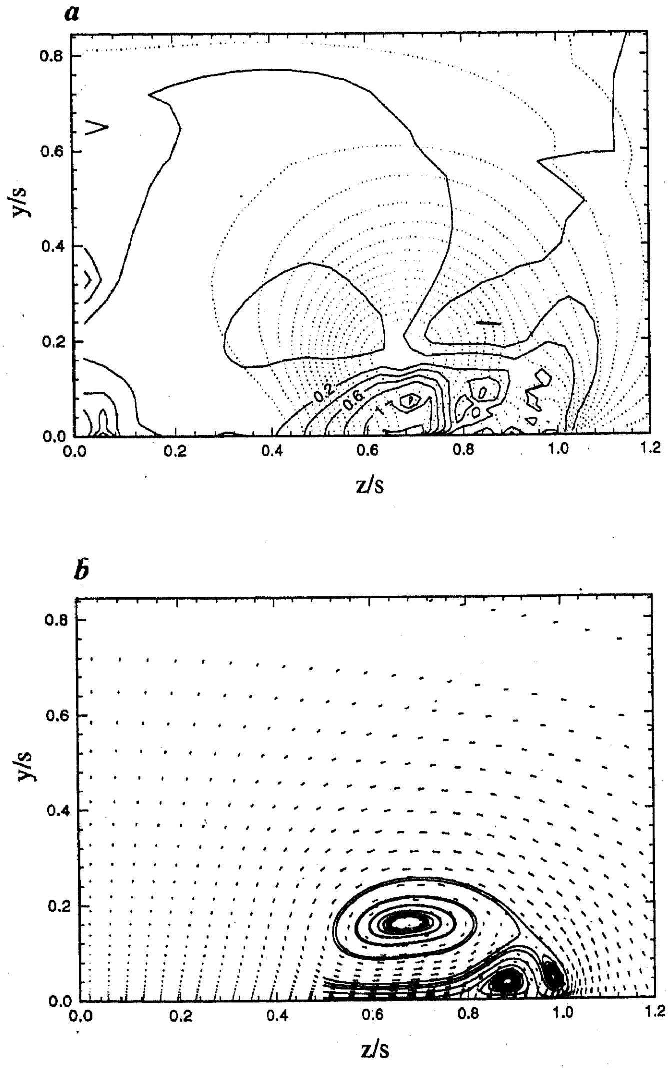

Figure 2. *a*, Contours of M_b along with C_p contours (dotted), and *b*, conically projected velocity vectors along with some streamlines, in the 1st TCP.

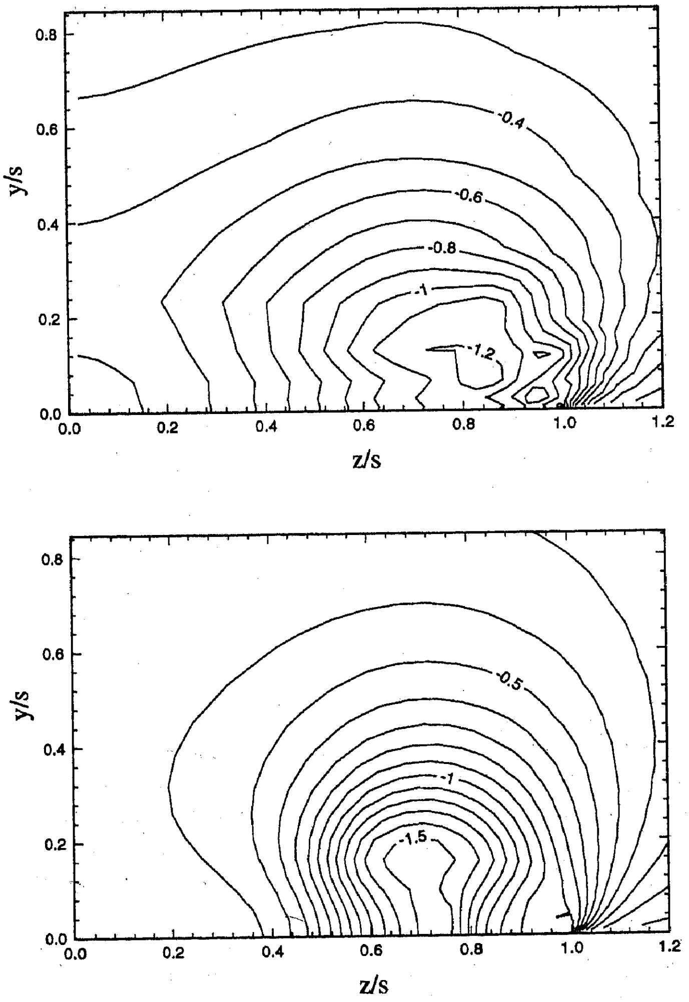

Figure 3. H–O solution C_p contours at $x = 0.02158$ (5th TCP), and at $x = 0.3048$ (19th TCP).

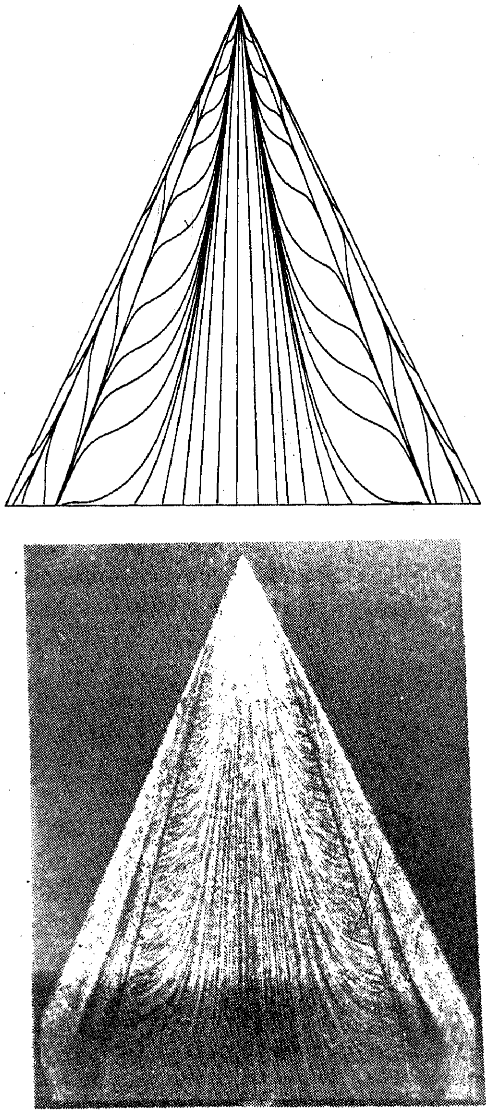

Figure 4. Computed and experimental lee-surface flow.

formed, as can be seen from the C_p contours in the 19th TCP. Compared to that in the 5th TCP, the suction in the vortex core in the 19th TCP is higher, and the C_p contours are just beginning to cluster immediately outboard of the foot of the vortex, indicating that a weak shock may be forming.

Figure 4 shows the lee-surface flow pattern given by the EC solution, and the experimental surface oil-flow visualization[2]. The computed near-conical surface-flow pattern is similar to that observed in the experiment, although it may be noted that the boundary layer in the experiment can modify the velocity vector orientation. A sharp change in the direction of the oil streak-lines and the computed surface streamlines near the secondary separation show the cross-flow shock. The cross-flow shock is seen to terminate the conically outboard flow and give rise to the secondary separation.

We have shown that an accurate simulation of the near-apex flow is necessary for the simulation of the cross-flow shock and, hence, the shock-induced secondary separation. By employing a grid, namely an embedded conical grid, that correctly takes into account the length scales of the flow considered, we have been able to capture, for the first time, the experimentally observed transonic vortex structure with embedded cross-flow shock and the shock-induced secondary vortex, right from the apex of the wing.

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Plasmid profile of *Erwinia herbicola* ATCC 21998

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Extra-chromosomal genome study of *Erwinia herbicola* ATCC 21998 was carried out. Two plasmids (pVQ1, pVQ2: mol. wt 7.4 and 8.0 kb respectively) were identified. One of the plasmids (pVQ2) was cured off and a restriction endonuclease map of the other plasmid (pVQ1) is established. *E. herbicola* ATCC 21998 being a keto-acid producing bacteria like *Gluconobacter oxydans* ATCC 9937 converts glucose to 2,5-diketo gluconic acid by a membrane-bound direct

glucose dehydrogenase system. Plasmid mediation of direct glucose oxidation in *G. oxydans* ATCC 9937 (refs 1, 2) prompted us to study the presence of glucose dehydrogenase gene on the plasmid of *E. herbicola*. Based on DNA/DNA hybridization it is suggested that glucose dehydrogenase gene is encoded in 4.3 kb *Sac* I fragment of plasmid pVQ1.

SEVERAL resident plasmids have been detected in almost all the classes of bacteria. It is also established that in several bacteria, plasmid genomes specify some of their characteristics. For example, in *E. coli*, location of genes that specify the production of enterotoxin, hemolysin, cell surface antigens (K antigen), etc. has been established on the plasmids[3]. Similarly many important determinants from the industrial viewpoint have been located on plasmids in *Pseudomonads*[4] and *Bacilli*[5]. However, in *Erwinia* species like *E. amylovora*[6], *E. chrysanthemi*[7,8], *E. herbicola*[7,8] and *E. stewartii*[9] none of the plasmids has yet been convincingly shown to be involved with specific primary or secondary function. Nevertheless, transmissible plasmids have been detected in human clinical isolates of *E. herbicola*[7,8]. One of these plasmids E-lac$^+$ carrying genes for lactose fermentation is transmissible albeit at a very low frequency to a variety of enterobacteria[7,8]. In pathogenic and non-pathogenic *Erwinia* species, several plasmid genomes have been detected. Some of these plasmids are reported as cryptic[10] whereas a good number of them are of significance[11,12].

E. herbicola ATCC 21998 is of immense industrial importance[13,14] because of its capability to generate partial and complete oxidation products (keto-acids) from glucose in high yields. However, due to lack of systematic studies on its genetic organization, basic aspects of its biology have not been elucidated so far. The studies on direct molecular cloning in *Erwinia* species have also been very limited[15–17] perhaps due to nonavailability of an appropriately efficient cloning vector. One of the prerequisites for the development of an efficient transformation system for a wild type organism is to know the natural resident plasmid genetics of the host. It was, therefore, considered desirable to study the natural plasmid genome of the wild organism. In this paper, data on plasmid DNA of the organism (*E. herbicola* ATCC 21998) is reported.

E. herbicola ATCC 21998 was obtained from American Type Culture Collection (USA). The organism was grown in ATCC 1038 medium[9]. Plasmid DNA was isolated from the organism by Birnboim and Dolly's[18] method with slight modifications (as described in the legend to Figure 1). The DNA was digested with various restriction endonucleases (2 U/µg) in appropriate buffer at 37°C for 8 h and analysed by electrophoresis on 1% agarose gel. The DNA bands were visualized on UV transilluminator after ethidium bromide staining. Using SEQAID software (SEQAID II (tm) version 2.20, Douglas D. Rhodes and Donald J. Roufa 1987, Kansas State University, Molecular Genetics Lab.) the sizes of all fragments were determined by comparing the mobility with that of the standard DNA size markers (Lambda-DNA digested with *Hin*dIII and *Pst*I, and ϕX 174-DNA digested with *Hinc*II). The map was prepared on CLONE III software (CLONE MANAGER – version 1.21 serial No. 31114 copyright 1988, scientific and educational software). DNA was transferred to nylon membrane (Amersham USA) following the method of Southern[19] and was fixed by UV cross-linking. DNA probes were prepared by labelling with non-radioactive digoxigenin (DIG) by random primer method (using Boehringer Manhiem kit and following its protocol). Hybridizations

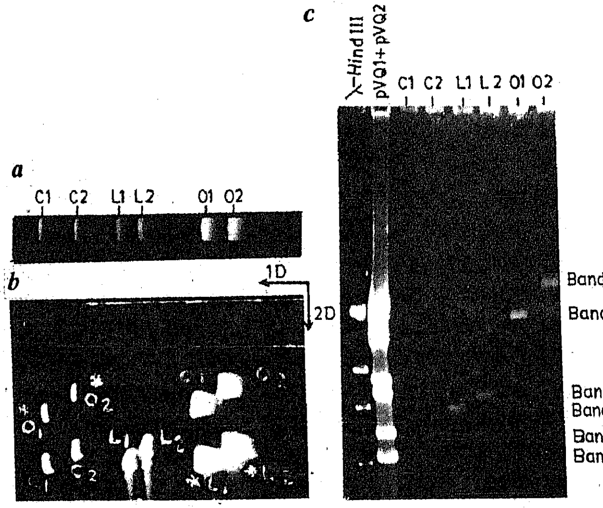

Figure 1. Plasmid DNA analysis of *E. herbicola* ATCC 21998. The cell pellet was washed with GTE buffer (50 mM glucose, 25 mM tris. Cl, 10 mM EDTA pH 8) and suspended in the same buffer with lysozyme (4 mg/ml). Incubated at RT for 5 min and then on ice for 2 min. the cell suspension was mixed gently with Sol II (0.2 N NaOH + 1% SDS) and incubated on ice for 10 min followed by addition of Sol III (5 M K acetate). After mixing and incubation on ice for 10 min the supernatant was collected by centrifuging at 10,000 rpm for 20 min. To the supernatant was added 5 ml of GES reagent (5 M guanidium thiocyanate, 100 mM EDTA and 0.5% Sarkosyl V/V pH 8). The mixture was incubated on ice for 1 h followed by chloroform extraction. Plasmid DNA was precipitated with two volumes of ethanol, washed with 70% ethanol, dried and dissolved in 10 mM tris, 1 mM EDTA. *a*, 1% agarose gel showing purified *Erwinia herbicola* plasmid DNA in different forms. *b*, Two-dimensional electrophoresis of 1% agarose gel showing conversion of CCC and OC form of plasmid DNA bands into their respective OC* and L* forms on exposure to UV radiation. *c*, 1% agarose gel showing different topoisomers of two plasmids. Lane 1, Lambda-*Hin*dIII marker; Lane 2, Various forms of plasmid DNA of *E. herbicola*; Lane 3, Eluted DNA band 1 (C1) showing partial conversion into its OC topoisomer (band 5); Lane 4, Eluted DNA band 2 (C2) showing partial conversion into its OC topoisomer (band 6); Lane 5, Eluted linear (L1) form of plasmid pVQ1; Lane 6, Eluted linear (L2) form of plasmid pVQ1; Lane 7, Eluted DNA band 5 showing partial conversion into its L topoisomer (band 3); Lane 8, Eluted DNA band 6 showing partial conversion into its L topoisomer (band 4).

and detections were carried out at 68°C under high stringent conditions (0.2 X SSC + 0.1% SDS) according to the supplier's protocol. Low melting agarose was obtained from FMC Sea Plaque (USA). Plasmid topoisomers were identified by the method of Hintermann *et al.*[20]. Plasmid curing was performed by using mitomycin C.

While analysing the chromosomal DNA of *E. herbicola* ATCC 21998 on 0.7% agarose gel, faint DNA bands were observed in the low molecular weight area, indicating the presence of plasmids. Plasmid DNA isolated from the mid-log phase culture when subjected to 1% agarose gel electrophoresis exhibited six bands within a wide migration range (Figure 1 *a*).

The presence of multiple bands indicated more than one resident plasmid in the organism. The two fastest migrating bands with highest electrophoretic mobility (C1, C2) could be taken for covalently closed circular (CCC) forms of two different plasmids. This was confirmed by two-dimensional agarose gel electrophoresis (Figure 1 *b*) in which the gel with total plasmid was irradiated with UV light at 254 nm for ten minutes before the second dimension to convert some of the CCC form of plasmids (C1, C2) into their open circular (OC) forms (O1*, O2*) and some of the original OC forms (O1, O2) into their linear forms (L1*, L2*). The newly formed OC bands (O1*, O2*) had the same slow mobility as that of the original OC forms (O1, O2). The originally present CCC (C1, C2) and OC (O1, O2) forms and the newly generated OC forms (O1*, O2*) form two triangles after electrophoresis in the second dimension showing the presence of two plasmids. The two additional bands formed by the original slowest migrating OC bands could be the linearized forms (L1*, L2*) which co-migrate with the two central linear forms (L1, L2) of the plasmid bands. This interpretation was confirmed by eluting out all the six bands on preparative scale from low melting agarose gel and re-run on 1% agarose gel (Figure 1 *c*) individually in each well. From Figure 1 *c* it is clear that the two plasmids having CCC forms as bands 1 and 2 give rise to their OC forms as bands 5 and 6 respectively, whereas the earlier OC forms (bands 5 and 6) give rise to their linear forms which move to band positions 3 and 4 respectively. The original linear forms of the two plasmids which appeared as bands 3 and 4 remained unchanged and did not give rise to any additional band. The two plasmids were named as pVQ1 and pVQ2.

In order to understand whether the keto-acid-producing activity of *E. herbicola* was due to the plasmid-borne genes, the organism was subjected to curing and the ketogenic activity of the cured isolates checked by monitoring the clearing zone formation due to acid production on agar indicator plates (ATCC 1038 agar medium + 1% bromocresol green). Minimal inhibitory concentration (MIC) for curing agent mitomycin C, as determined by viable cell count method using broth dilution and plating out techniques, was found to be 6 µg/ml for *E. herbicola* ATCC 21998; at that concentration over 99.99% kill was achieved. Approximately 500 mitomycin C-treated colonies were screened for loss, if any, of plasmid DNA. Three colonies showed the presence of only one plasmid pVQ1 in which the other plasmid pVQ2 had cured off. The presence of only one plasmid pVQ1 and curing of pVQ2 was confirmed by hybridization using DIG-labelled pVQ1 and pVQ2 DNA probes. The cured isolate of *E. herbicola* exhibited the presence of plasmid pVQ1 only.

For characterization of plasmid pVQ1 at molecular level, the DNA was subjected to restriction endonuclease digestion analysis. The positions of the cleavage sites for restriction enzymes and the molecular sizes of the fragments were determined by analysis of single, double and triple digestion products fractioned on agarose gels.

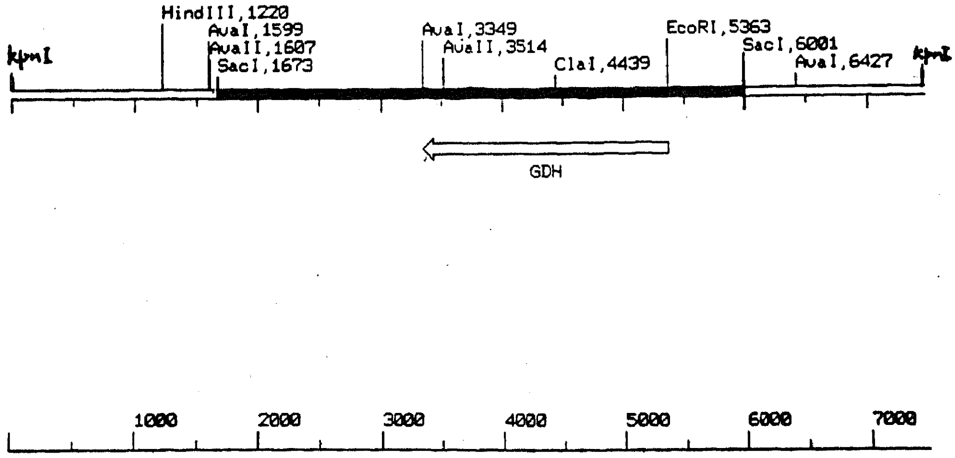

Figure 2. Restriction map of plasmid pVQ1 showing position and map of putative *gdh* gene.

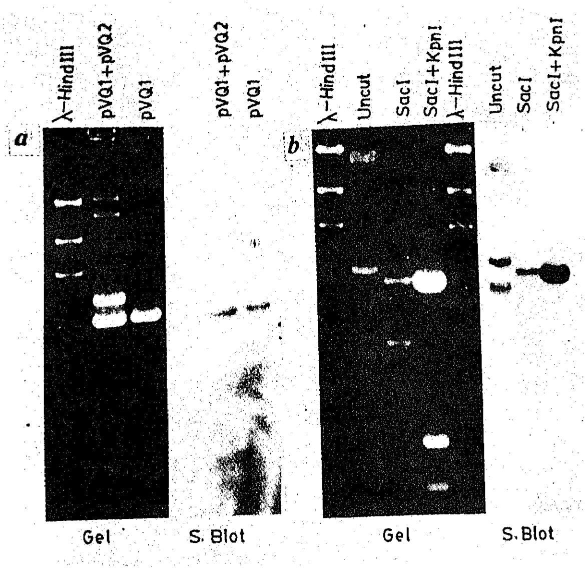

Figure 3. Identification of *gdh* gene on plasmid pVQ1 and its location on 4.3 kb *Sac*I fragment. C-terminal end was isolated from the *gdh* gene of *A. calcoaceticus* and labelled with digoxigenin by random primer method. The probe was hybridized with the Southern blots of gels (*a*) containing both plasmids pVQ1 and pVQ2 from wild *E. herbicola ATCC 21998* and only plasmid pVQ1 from cured isolate and (*b*) containing plasmid pVQ1 cut with *Sac*I and *Sac*I + *Kpn*I. The hybridization was carried out at 68°C over night. Blots were washed under high stringent conditions and detection was done with NBT/X-phosphate.

The molecular weight of the plasmid pVQ1 was estimated to be about 7.467 kb. The restriction map of the plasmid as shown in Figure 2 indicates that the plasmid possesses unique restriction site for *Eco*RI, *Hin*dIII, *Kpn*I and *Cla*I and two sites for *Sac*I, and *Ava*II, and three sites for *Ava*I. However, no restriction sites for *Bam*HI and *Sph*I were found on the plasmid.

When the C-terminal end conserved DNA sequence of glucose dehydrogenase (*gdh*) gene of *Acinetobacter calcoaceticus* was used as a probe and hybridized against the total plasmid DNA of *E. herbicola*, pVQ1 got hybridized (Figure 3 *a*), giving strong indication that the plasmid bears the *gdh* gene. The hybridization was traced to its 4.3 kb *Sac*I fragment (Figure 3 *b*). The restriction map of the putative *gdh* gene-bearing fragment is shown in Figure 2. The fragment possesses single restriction site each for *Ava*I, *Ava*II, *Cla*I and *Eco*RI. Digestion with these restriction enzymes produced fragments of (2.7 kb and 1.6 kb), (2.5 kb and 1.8 kb), (2.8 kb and 1.5 kb) and (4.1 kb and 0.2 kb) respectively. *Hae*III and *Hpa*II generate multiple restriction sites. The fragment was cloned in *E. coli* SURE^{TM} using vector pUC18 and attempts are underway to express the putative *gdh* gene.

The above findings suggest that further in-depth study of extra-chromosomal genetics of *E. herbicola* ATCC 21998 may help us in understanding the role of plasmid pVQ1 in direct glucose oxidation pathway in the organism. Moreover, this plasmid is also of interest because of its possible use for the development of a cloning vector for which assessment of its transformation and conjugation efficiency are being made.

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Satellite data reveals pre-earthquake thermal anomalies in Killari area, Maharashtra

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The analysis of satellite thermal infrared data obtained from Landsat-5 Thematic Mapper (TM) of Killari area for pre- and post-earthquake periods has indicated thermal anomalies (building up of temperature) prior to the earthquake. These thermal anomalies are associated with the major lineaments and match well with the locations of geothermal activity in the area reported immediately after the earthquake.

EARTHQUAKES are the natural disasters that kill thousands of people, destroy property and leave a long lasting impact on the environment. But, the forecasting and on-time warning of earthquakes remain one of the most important problems of earth and space sciences[1,2]. During the last couple of decades, studies have shown anomalous physical phenomena prior to the earthquake, which include various effects of heat, sound, light, electricity, magnetism, atmosphere and so on[3]. The sensors on-board space platforms provide appropriate information on some of these phenomena. Detection of thermal anomalies prior to the earthquake is one of the potential areas wherein remote sensing satellites play a significant role. Currently, the data obtained from remote sensing satellites are mainly used to identify and map the seismic zones[4,5] and to locate safer places for constructing the villages[6]. However, the studies related to earthquake precursors using satellite thermal images are still at infancy stage.

The study of pre-seismic geothermal anomalies is an important subject matter in earthquake-prediction research. According to Ma Zongjin *et al.*[3], all the nine major earthquakes of China, which took place between 1966 and 1976, were invariably associated with thermal anomalies of various kinds, such as heating of the ground (20–80 cm depth), melting of ice, increase in water temperature of springs by 10°C and sultriness felt by people. By comparing and analysing the main thermal anomalies before the nine major earthquakes and local weather variation data, they have concluded that in five of the nine earthquakes, the output of underground heat could not be discounted. According to Gold and Soter[7], major earthquakes are often preceded and/or accompanied by phenomena related to eruption of high pressure gas at the sites of magmatic activity in the form of heating of the ground, smell of sulphur or hydrocarbons, geosound and turbulence/bubbling in groundwater, rivers and lakes (from Rastogi and Rao[8]). However, the reasons for such pre-seismic thermal phenomena in non-volcanic terrain are not clear. Scholz *et al.*[9] and Sibson *et al.*[10] have proposed the dilatancy-diffusion model to explain migration of hydrothermal fluids through fractured rocks before and after the earthquake (from Chandrasekharam[11]).

The Killari earthquake of 30 September 1993, which jolted parts of Latur and Osmanabad districts of Maharashtra, was followed by post-earthquake geothermal activity in the form of (i) smoke/gas emanations from the ground/bore wells/water bodies, and (ii) heating of the ground and water in the bore wells/springs. This geothermal activity was reported from various places as far as 200 km away from the epicentre in the States of Maharashtra, Andhra Pradesh and Karnataka, and continued for about 3–4 weeks after the earthquake[6,8,11–15]. Figure 1 shows one such live smoke/water vapour emanation activity at Kothapally village, located 24 km north of Nizamabad town, AP. This activity was confined to an area of about 1.5 m radius. Temperature measurement carried out using digital thermal infrared radiometer has indicated that the temperature at the site of this activity was 42°C against the background temperature of 33°C and it went on increasing substantially with depth (about 10°C per ft). At 1 ft depth, the temperature was 52°C, at 2 ft depth it was 62°C and at 3 ft depth it became 70°C (ref. 6). Similar geothermal events in the form of steam emanation from ground at Bhatangli and gas bubbling in a lake and an open dug well at Warud were observed by Chandrasekharam[11]. It is quite likely that these geothermal anomalies were the manifestations of pre-earthquake warming which went unnoticed. Keeping this in view, a study has been carried out to detect the presence of pre-earthquake thermal anomalies, if any, using spaceborne thermal infrared data obtained from Landsat-5 TM.

Landsat-5 TM band 6, operating in thermal infrared region (10.4–12.5 μm) of electromagnetic spectrum, provide thermal (temperature) images of earth's surface once in every 16 days with the spatial resolution of 120 m and radiometric resolution of 0.5 K. The ground temperature measured by the satellites depends upon the solar heating, surface thermal properties, meteorological and near surface atmospheric conditions as well as on processes occurring in the upper crust of the earth. It is the latter which is relevant for studying the thermal anomalies in seismoactive regions[16].

For studying the pre-earthquake thermal anomalies in Killari area, two data sets of Landsat TM pertaining to 20.09.93 (pre-earthquake) and 06.10.93 (post-earthquake) periods covering about 7,000 sq km around the epicentre have been acquired and processed. Initially, both the data sets have been geometrically registered, and the thermal infrared (band 6) data have been normalized for changes in solar elevations and atmospheric condi-

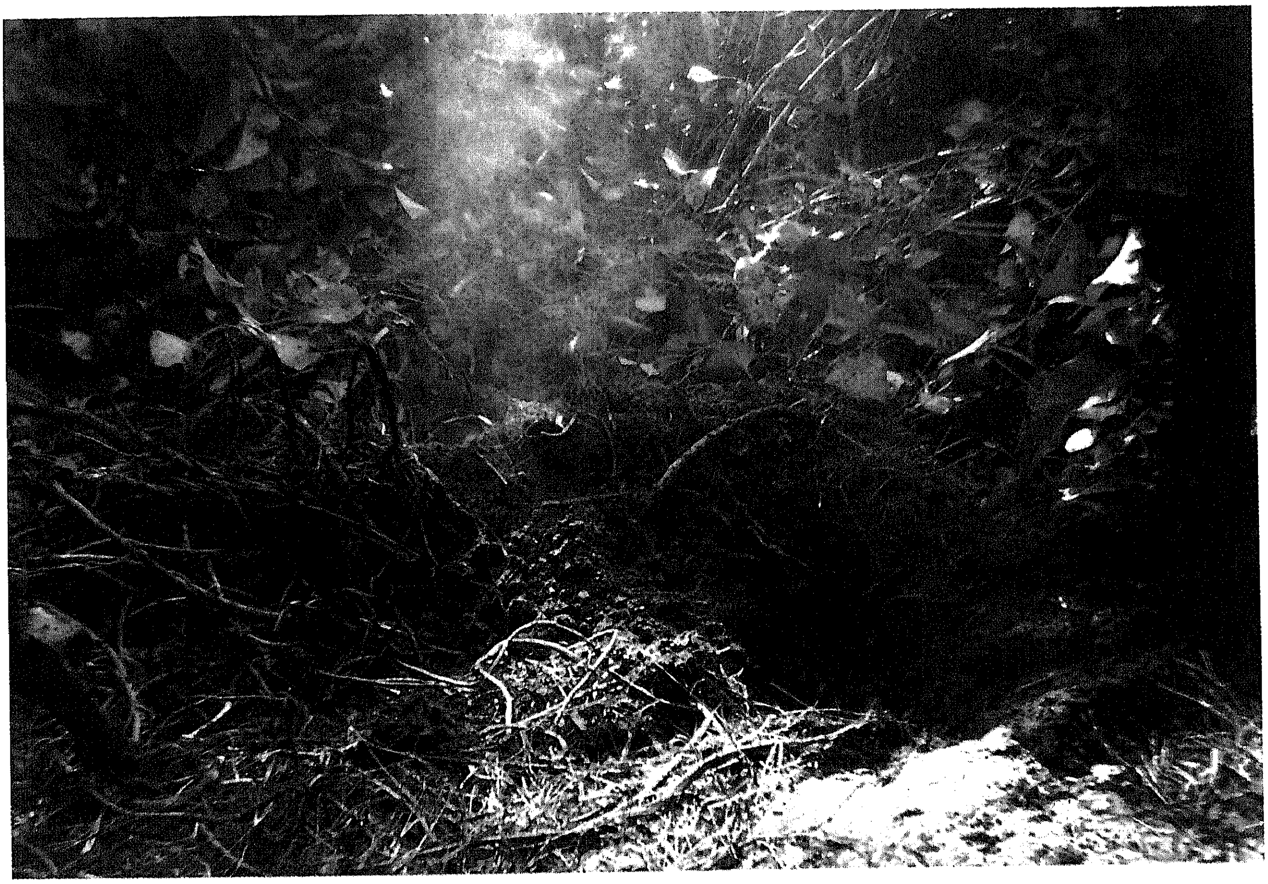

Figure 1. Field photograph showing smoke/water vapour emanation at Kothapally village (Courtesy: K. Seshadri).

tions. The atmospheric corrections were done by taking water bodies in both the images as common features and band 6 DN (Digital Number) values were normalized taking post-earthquake data as reference. Image differencing of normalized band 6 of both the dates (pre–post) after removing the noise has brought out the pre-earthquake thermal anomalies, indicating building up of temperature prior to the earthquake. In other words, DN values of the pre-earthquake data are higher than that of post-earthquake at the locations of observed thermal anomalies. Since, there is only 16 days difference between the two data sets and the land use is the same as seen on satellite data at the places of observed thermal anomalies in both the dates, the thermal anomalies could not have arisen from any other imaginable cause.

Figure 2 shows the false colour composite (FCC) of Killari area (band 4 (0.76–0.90 μm) = red, band 3 (0.63–0.69 μm) = green, band 6 difference (pre–post) = blue) depicting the thermal anomalies in blue colour. Figure 3 shows the pseudo-colour image of Killari area indicating thermal anomalies in red against the background in cyan. The major lineaments interpreted from satellite image and locations of reported smoke/gas emanations are also shown on these images. The pre-earthquake thermal anomalies which are shown in blue in Figure 2 and in red in Figure 3 are observed to match well with the reported locations of geothermal activity, such as Bendkal, Rui and Takli. Though, smoke emanation from ground is reported at Nilanga and Umarga, the pre-earthquake thermal anomalies are found on their southern and western sides, respectively. The imagery also show several spots with thermal anomalies where post-earthquake geothermal activity is not reported. It is important to note that the observed thermal anomalies are pre-seismic and it is not necessary that at all the places, post-earthquake geothermal activity will be reported. The individual spots of thermal anomaly only indicate raising of ground temperature due to underground heat in the surrounding area selectively as a precursor of the present earthquake. Interestingly, the thermal anomalies also show correlation with the major lineaments existing in the area. This is in corroboration with the dilatancy-diffusion model[9,10] which explains the physical basis of pre- and post-earthquake geothermal activity along the dilatant/lineament zones.

The source of underground heat causing the thermal anomalies on the surface is not clear. The onset of

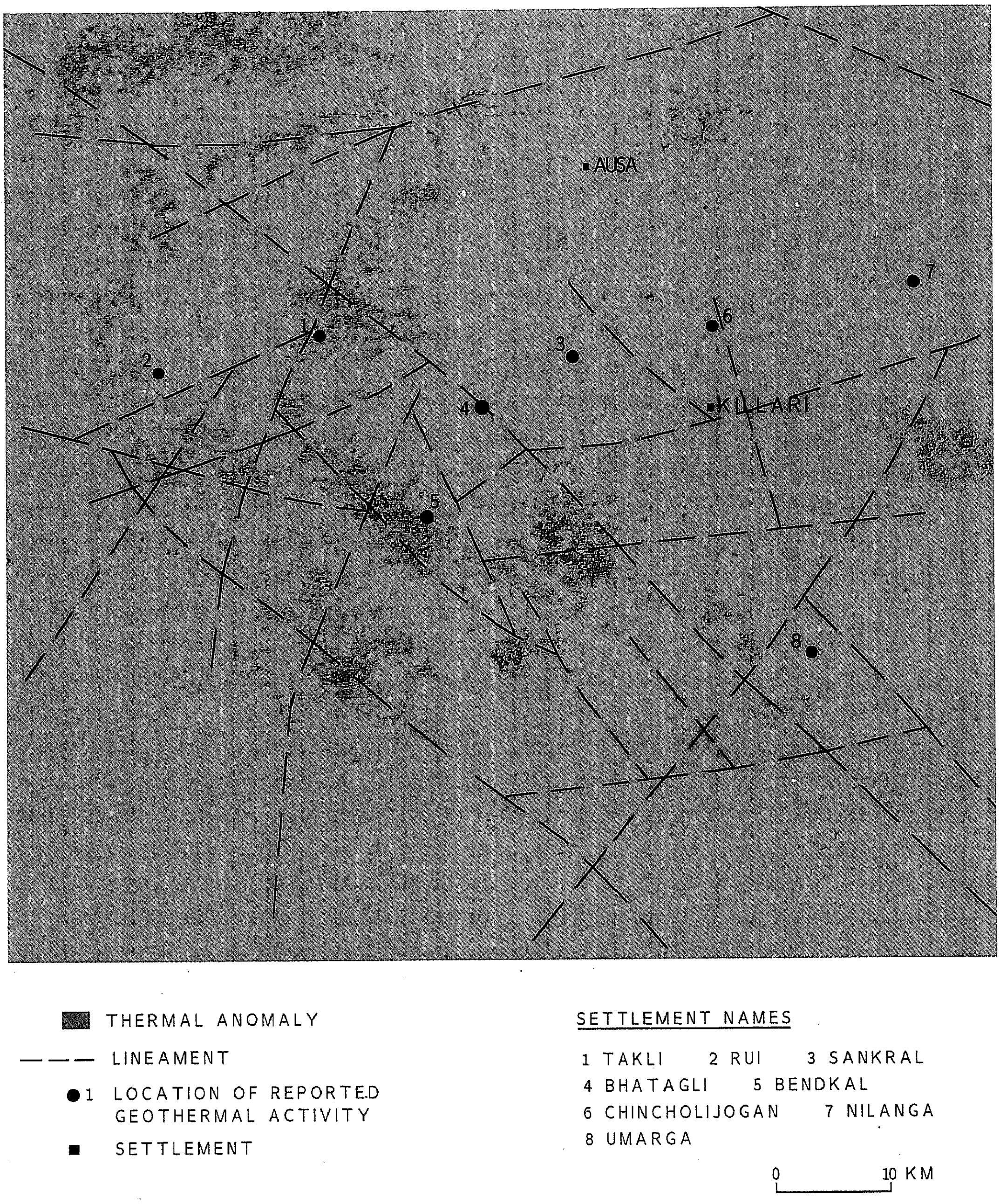

Figure 2. TM FCC of Killari area depicting thermal anomalies in blue (band 4 = red, band 3 = green, band 6 difference = blue).

fresh volcanic activity has been discounted[8,15]. Chemical analysis and silica thermometry of water samples, collected in the epicentral zone and in the surrounding areas where smoke/gas emanation and heating of the ground are reported, suggest the inflow of geothermal fluids from deep reservoir to the ground level[13]. Chandrasekharam[11] advocates seismic pumping mechanism as the reason for geothermal activity. According to him, the load pressure and compressional forces due to northerly movement of Indian plate would have squeezed out fluids from the surrounding Bhima sedimentary rocks. Due to prevailing pressure difference between the source region of these fluids and the dilatant zones, hot fluids (considering the focal depth of earthquake and normal geothermal gradient) entered or sucked into these zones prior to the main shock and later got ejected as steam on the surface. Therefore, it is quite likely that the pre-earthquake thermal anomalies detected through satellite data were caused by the presence of hot fluids/gases along the lineaments which were released subsequently as steam/smoke due to opening of cracks/fissures after the earthquake.

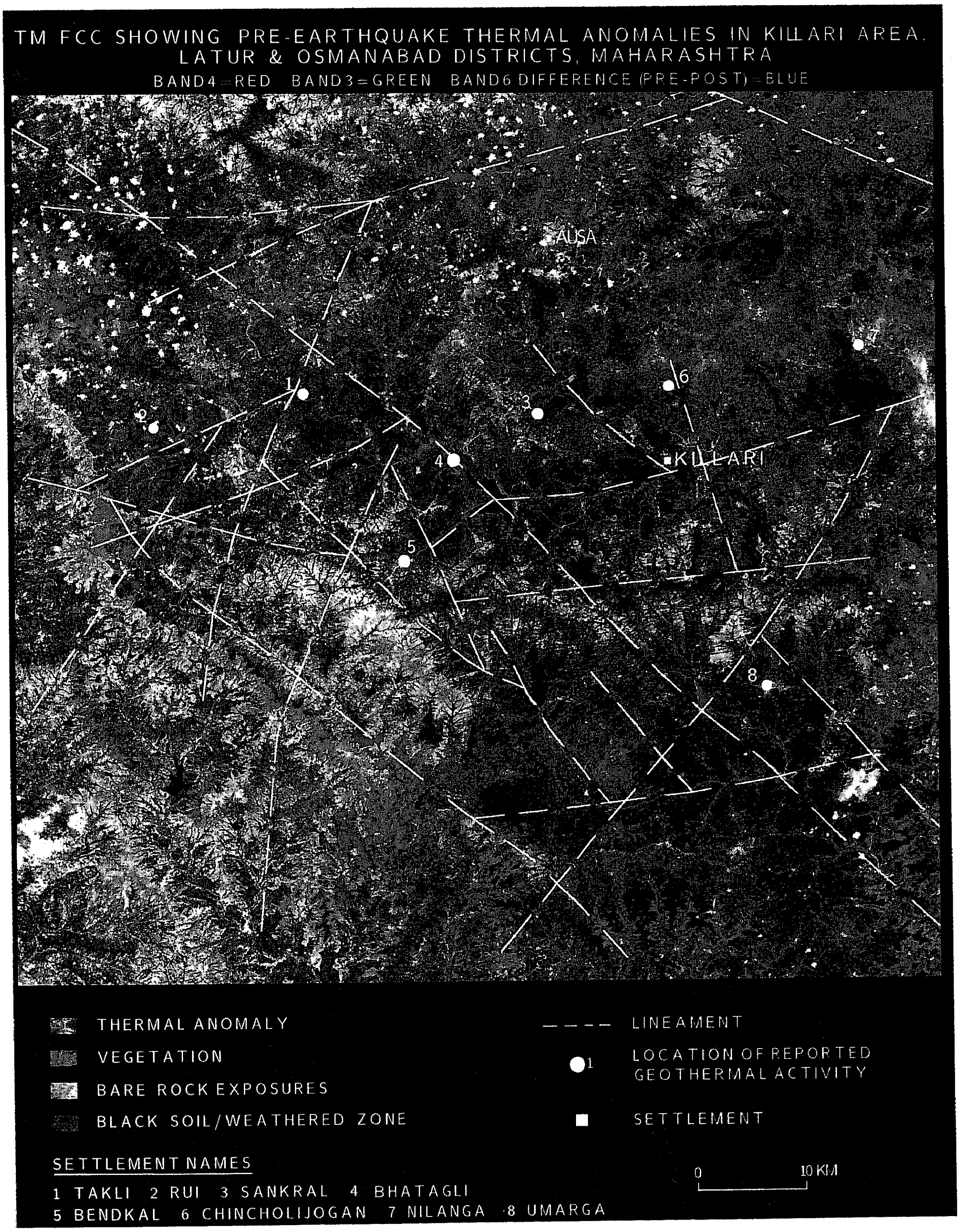
TM FCC SHOWING PRE-EARTHQUAKE THERMAL ANOMALIES IN KILLARI AREA, LATUR & OSMANABAD DISTRICTS, MAHARASHTRA
BAND4 = RED BAND3 = GREEN BAND6 DIFFERENCE (PRE-POST) = BLUE
AUSA
KILLARI
1
2
3
4
5
6
8
THERMAL ANOMALY
VEGETATION
BARE ROCK EXPOSURES
BLACK SOIL/WEATHERED ZONE
LINEAMENT
LOCATION OF REPORTED GEOTHERMAL ACTIVITY
SETTLEMENT
SETTLEMENT NAMES
1 TAKLI 2 RUI 3 SANKRAL 4 BHATAGLI
5 BENDKAL 6 CHINCHOLIJOGAN 7 NILANGA 8 UMARGA
0
10 KM

Figure 3. Pseudo-colour image of Killari area depicting thermal anomalies in red.

In the present study, two sets of satellite data (one 10 days prior to the earthquake and another 6 days after the earthquake) have been analysed, indicating that the thermal anomalies became noticeable at least 10 days before the earthquake took place. However, it has not been possible to establish how long before the Killari earthquake, the thermal anomalies in the area appeared because of the long repetivity (16 days in case of Landsat) of high (fine) resolution satellite, absence of thermal infrared sensor on IRS-series of satellites and non-availability of cloud-free images. TM thermal infrared data of 06.12.92, 24.02.93 and 31.05.93 have not revealed any thermal anomaly in the area, and the temperature variation observed in different periods was due to seasonal effect and changes in land use pattern. The study carried out in Central Asian Seismoactive region, Iran, Egypt, etc. using the National Oceanic and Atmospheric Administration (NOAA)-series satellite thermal images (10,000 numbers over a span of ten years) revealed positive infrared (IR) anomalies of few degree centigrade over large scale faults, and statistically significant correlation between the activity of IR anomalies and the seismic activity[16].

This study highlights the potential of satellite data for identifying thermal anomalies which may be used as precursors to earthquake. However, more intensive studies have to be carried out in other seismically active areas for establishing a relation between the appearance of the thermal anomalies and impending earthquakes.

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Fission track dating of obsidian artefacts from Columbia

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The fission track technique is useful for dating young obsidian artefacts owing to their high uranium content. It is being used for obsidian source identification and provenance studies. Fission track age of Columbian obsidian from an archaeological site, Rio Hondo near Popayan, is found to be 3.83 Ma.

THE fission track technique is found to be most suitable for dating natural glasses[1-10], namely, obsidians and tektites owing to their high uranium content despite certain limitations. Volcanic glasses commonly present two particular problems to fission trackologists: they have microlites and gas bubbles which, after etching, are easily confused with fission tracks. Another limitation is the thermal heating of obsidian artefacts, which results in track fading, thus yielding reduced fission track ages of source material. Obsidian artefacts from archaeological sites around the world are dated for their source identification. The purpose of this study is to determine the fission track age of obsidian artefacts discovered at Rio Hondo, near Popayan in Columbia. The samples were analysed by coupling of PIXE and fission trace dating for trace element analysis and source identification at Grenoble[11]. Identifying the source of obsidians often directly provides evidence of pre-historic human activity, trade routes and cultural communication that took place between the archaeological site and the obsidian source area[12].

Experimental technique used for preparation, etching, irradiation and counting of obsidian samples is the standard one described elsewhere[9-10]. The obsidian artefact samples were broken, polished and etched in 6% HF for 8 min at room temperature to develop the fossil tracks. The counting of fossil tracks is a tedious job and the first two authors performed their independent counting after polishing and etching a freshly cleaved surface. The individual counting differs by as much as 10% which is also the range of statistical counting error. The samples were annealed in a furnace at 500°C for 1 h to remove fossil tracks before irradiation in CIRUS reactor at BARC, Trombay. Both the annealed obsidian samples and a standard glass wafer (NBS-SRM 962, U content 37.38 ± 0.08 ppm) were irradiated simultaneously in a thermal neutron fluence of 10^{15} n/cm^2. Irradiated samples were etched under identical conditions as used for original batch samples for fossil tracks. The track density for both fossil and induced tracks was determined using a calibrated graticule in the Carl Zeiss binocular

Table 1. Fission track ages of Rio Hondo obsidians from Columbia (Thermal neutron fluence $F = 3.512 \times 10^{15}$ n/cm^2)

	Track density (per cm$^2 \times 10^4$)		
Sample	ρ_s (n_s)	ρ_i (n_i)	Fission track age (Ma) ± 1σ
RH-1	0.573 (85)	31.25 (821)	3.87 ± 0.42
RH-2	0.559 (79)	30.87 (736)	3.82 ± 0.42
RH-3	0.587 (90)	32.69 (756)	3.79 ± 0.40

Mean fission track age = 3.83 ± 0.42 Ma (Figures in parentheses show the number of tracks counted).

microscope. The track density in the standard glass dosimeter was used to estimate the neutron fluence.

Fission track ages are calculated using the simplified version of age equation[13]

$$T = \frac{\rho_s \sigma \, \mathrm{IF}}{\rho_i \lambda_f}, \qquad (1)$$

which reduces, on substitution of values of σ (582×10^{-24} cm^2), I(7.26×10^{-3}) and λ_f (7.03×10^{-17} yr^{-1})[14], to

$$T = 6.01 \times 10^{-8} \frac{\rho_s}{\rho_i} \mathrm{F}, \qquad (2)$$

where ρ_s and ρ_i denote recorded fossil and induced track densities respectively and F, thermal neutron fluence during irradiation.

Fission track ages of obsidian artefacts from Rio Hondo archaeological site near Popayan in Columbia are listed in Table 1. The statistical counting error, 1σ, calculated on the basis of fossil track counts is also given. The mean fission track age value of 3.83 Ma agrees well with the source age 3.70 Ma determined for this site[11]. It is obvious that the fission track technique combined with PIXE can prove useful for source identification and provenance studies of obsidian artefacts.

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Asian elephants with longer tusks have lower parasite loads

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The Hamilton and Zuk hypothesis[1] that the intensity of male ornamentation allows females to assess a male's ability to resist parasites has been much debated recently[2–12]. Much of the empirical work to test this hypothesis has been with insect[2], fish[3,4], reptilian[5] or avian[6–9] hosts. In a southern Indian population, we show that the length of tusks of male Asian elephants (*Elephas maximus*), corrected for differences due to age, is significantly negatively correlated with intestinal parasite loads. The less aggregated distribution of parasites in this elephant population, as compared to other mammalian species, indicates that ivory poaching may have already selectively removed a significant proportion of parasite-resistant individuals. Ivory poaching which targets larger-tusked elephants may thus affect the health status of the population.

FREELAND[13] suggested that mate choice by females may be related to parasites and pathogens carried by males. Hamilton and Zuk[1] further developed the hypothesis that host-parasite coevolutionary cycles might drive the evolution of female preferences for extreme male displays. Males carrying genes for resistance to parasites will be healthier and in a better condition to develop expensive secondary sexual characters. By preferring such males, a female ensures 'good genes' for her offspring. Unlike other 'good gene' hypotheses, however, the resistance genes will be constantly changing since the parasites are also evolving, and thus the system remains dynamic maintaining the importance of female choice.

Negative correlations between parasite loads and secondary sexual characters have been shown in invertebrate, fish, reptilian and avian systems[2–9]. In a mammalian system, Folstad *et al.*[14] found that experimental removal of parasites, through antihelminthic treatment, in female reindeer (*Rangifer tarandus*) did not increase antler length but increased antler symmetry. We have examined the length of tusks in male Asian elephants in relation to their intestinal parasite loads.

We have been studying the ecology of elephants in southern India since 1980[15]. Our study on mammalian parasites[16,17] was carried out in the Mudumalai Wildlife Sanctuary, a 321 km^2 reserve which is part of a much larger elephant range in southern India. The vegetation in Mudumalai changes from tropical moist deciduous forest in the west through dry deciduous forest to dry thorn forest in the east, corresponding to a gradient in rainfall from 1800 mm to about 700 mm per annum. The density of elephants in the reserve is about 3 individuals/km^2 (ref. 18). As with most other reserves in southern India, the elephants of Mudumalai have also suffered from poaching of males for ivory[15]. Consequently the ratio of adult (> 15 years) male to female has changed from about 1 : 5 in 1981 to about 1 : 15 in 1996 (unpublished data).

Wild elephants at Mudumalai were sampled by collecting fresh defecations during 1990–92 and 1994–95. Elephants were identified, whenever possible, based on the characteristics of their tusks, ears, tails and other prominent marks, and photographs of side profiles were taken for measuring tusk length. These elephants were followed until fresh dung could be collected. The approximate age of the animal was determined by its estimated height, ear foldings, and thickness of the tusk at its base[19]. The photographs were enlarged for better resolution, and the actual tusk length along the curves determined by comparison to the height of the animals.

Densities of helminth propagules in the faecal samples were determined by a quantitative modification of the sedimentation flotation method. Parasite ova from weighed quantities of dung samples were concentrated by zinc sulphate density gradient centrifugation and all the ova floating to the top counted[16,17]. Elephants in the study area showed a high prevalence of parasites as compared to other herbivorous mammals of the study area, although the species richness was low[17]. The parasite fauna was dominated by nematodes of the genera *Quilonia*, *Murshidia* and *Decrusia*. The estimated faecal output of nematode eggs per day varied from zero to twenty million. There was a significant difference in parasite loads in the elephant population between seasons, with the dry season loads being significantly higher than the wet season loads (median loads of 25 and 9 respectively; $n1 = 39$, $n2 = 60$, $T = 1547$, $p < 0.01$). Since the faecal samples from bull elephants were also collected in different seasons, the seasonal differences were controlled by subtracting the seasonal and overall population median difference from each of the samples.

Tusks grow throughout the life of a male Asian elephant, although the rate of growth slows down after 30 years[19]. We constructed a standard growth curve of tusks (Figure 1) by the following procedure. For a given population, the girth of tusks of an individual is known to be a reliable indicator of age[19,20]. The tusk length, on the other hand, is much more variable. The relationship between circumference of tusks at the lip line (TC) and age (t in years) is given by: $TC_t = 43.4 \{1 - \exp[-0.064t]\}$ cm for Asian elephants in southern India[18]. We took measurements of tusk length (TL) and tusk circumference (TC) of 158 pairs of tusks from museum specimens and post mortem examinations of wild elephants. After determining age from circumference, we plotted tusk length versus its estimated age (Figure 1). The following equation offered the best fit to the data, $TL\ (\text{cm}) = -57 + 130 \log(t)$

The deviation in tusk length of individual wild bulls from the standard curve was plotted against the parasite propagule densities (Figure 2). Faecal samples from 38 tuskers (> 10 years of age) representing at least 26 distinctly identified bulls were analysed. Two of these identified bulls were sampled more than once. Some of the unidentified bulls may have been different individuals,

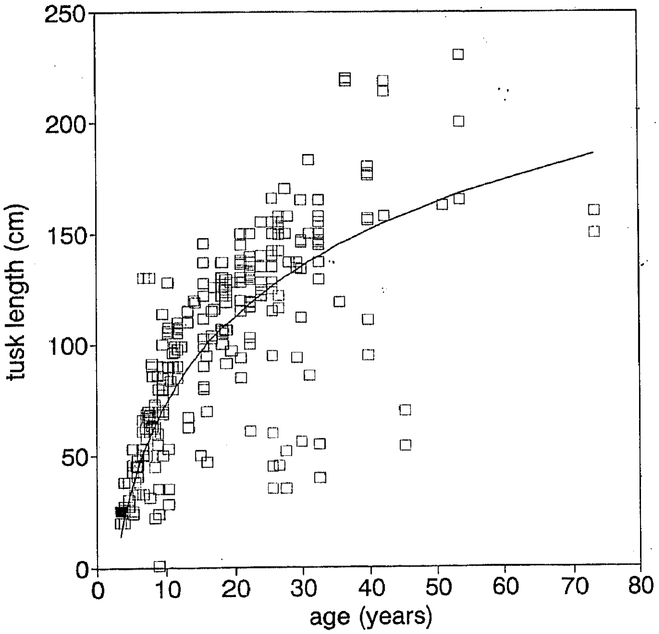

Figure 1. Tusk length in male Asian elephants as a function of age. An equation for the best fit line was obtained by fitting a linear regression of tusk length on a log transform of age ($r = 0.78$). The equation gives a curve on a linear axis. This curve was used as the expected tusk length for a given age class. The modal tusk length for each age class is above this curve. A number of individuals having substantially smaller tusks tend to pull down the best fit line.

while others may have been sampled repeatedly. A significant negative correlation between tusk length deviation and parasite density was obtained using Kendall's Tau (Tau = – 0.255, $p < 0.01$). Statistical significance is not lost by omitting data from repeated sampling of identified bulls. Our analysis did not include tuskless bulls.

The correlation is compatible with, although not necessarily a substantial proof of, the Hamilton and Zuk hypothesis[1]. Tusk growth may be affected by genetic as well as environmental variables and our data suggest that parasite loads may be one of the important factors. One of the limitations of our data is that they represent a one time sampling for parasite loads, which may not reflect the real parasite burden experienced by the bull during the entire period of tusk growth. It is also likely that parasite loads during a certain age is more important for tusk growth. We observed, however, that two of the identified animals that were sampled repeatedly over a period of five years showed much lower variation (SD = 11.0) in parasite propagule densities than the population variation (SD = 33.3). One time sampling of other animals therefore can be considered fairly representative.

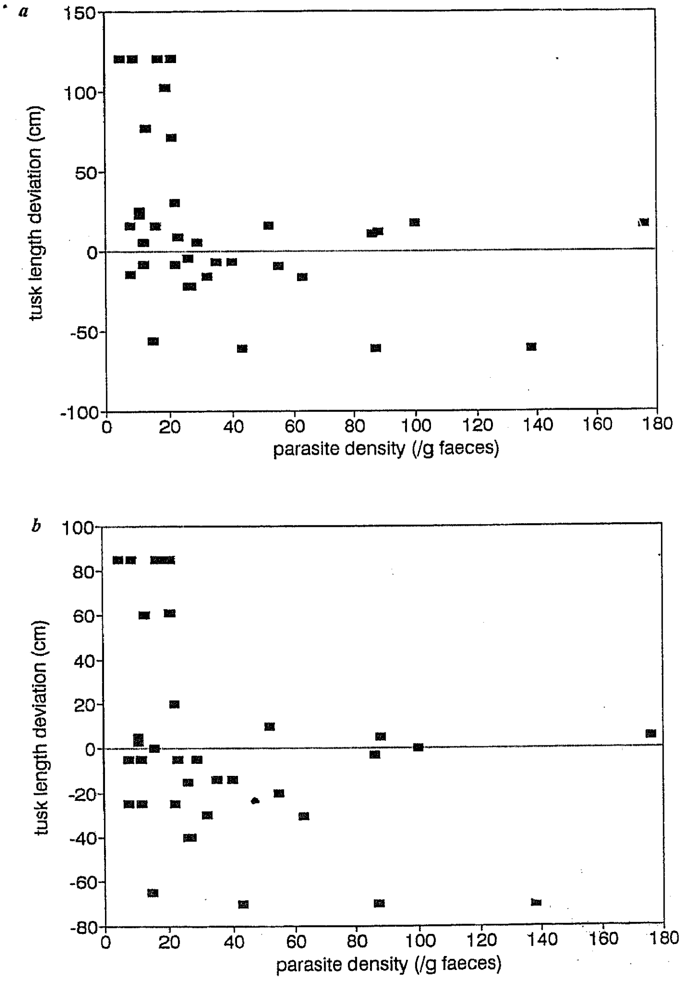

Figure 2. Correlation between tusk length deviation (from the expected) and parasite loads in male elephants. ***a***, Deviation from the best fit line. ***b***, Deviation from the modal tusk length.

The extreme outlier on the right was a bull in *musth* condition for at least three weeks before sampling. The food intake reduces considerably during *musth* resulting in an apparent increase in density of parasite ova. The negative correlation is significant including this individual. A possible correction for *musth* condition will shift the point to the left, strengthening the correlation further.

Earlier works on parasite loads in relation to sexual selection, have considered different types of parasites including blood parasites[1,21], coccidians[22], ciliates[4] and gregarines[2]. These parasites multiply for several generations within the host body. Only certain types of parasites can be involved in coevolutionary cycles, leading to sexual showiness. Parasites ideally suited are of the medium virulence, chronic type which are likely to produce coevolutionary cycles long enough to create average positive parent–offspring fitness correlations[1]. Too short a cycle may produce negative correlations, while parasites that can multiply within a host individual will probably evolve too fast and may not be suitable for this model. The external life-cycle parasites such as the visceral helminths, on the other hand, must have life cycle stages outside the host that would invade any host individual again. For such parasites, selective pressures will operate to overcome not the genotype of an individual host but the most prevalent genotype in the host population. These are the parasites expected to lead to coevolutionary cycles of appropriate duration and therefore should be the parasite group of choice.

Thomas *et al.*[23] point to the possibility of a negative correlation between parasite loads and male showiness being a fallacy. Parasitism can be negatively correlated with host age while male showiness and female choice covary positively with age. This bias is unlikely in our data because we considered the deviation from expected tusk length for a given age, and also because elephants parasite loads in the study area did not vary significantly with age. For a sample of 184 elephants of all ages and both sexes tested during 1990–92 in the study area, no relationship was observed between dung bolus diameter (a simple measure of age class) and parasite loads[16].

The ecological and evolutionary role of tusks in elephants is debatable. Tusks may play a role in obtaining nutrition (debarking of trees, for instance), in defense against predators, in male–male competition, and may also act as a secondary sexual character influencing female choice of mates. Even when both the sexes possess tusks, as in the African elephant (*Loxodonta africana*) or the extinct mastodon (*Mammut americanum*), there is sexual dimorphism for tusk size with the males having larger tusks than do females of corresponding age[20,24]. Beyond a certain size, the tusks may also become a handicap (heavy, long tusks may slow the mobility of the individual, while tusks which cross at their tips may actually hamper the use of the trunk in feeding). The tusks can thus be potential candidates for Zahavi's 'handicap principle'[25], according to which females may actually prefer males exhibiting a 'handicap', because an exaggerated secondary sexual character which reduces a male's chances of survival also acts as a signal of genetic quality. In elephants the question as to whether females choose males with longer tusks still remains unanswered. Female African elephants are known to choose larger and older bulls[26,27] and older bulls generally have longer tusks[20,28]. A similar choice may exist for longer tusked bulls in a given age class. Our data show that, while short-tusked males may have either low or high parasite loads, the long-tusked males almost invariably have low parasite loads. Females choosing long-tusked males would thus ensure a parasite-resistant mate.

A negative correlation between tusk length and parasite loads also has implications for conservation. The high incidence of poaching of African elephants[29,30] and Asian elephants[15] in recent decades may thus have made impacts more serious than just lowering total population sizes[29] or genetically effective population sizes[15,31], causing demographic changes, or randomly eroding the genetic variation in a population. Ivory poachers tend to preferentially target elephants with longer tusks and in the process may be selectively eliminating genes for resistance to parasites or diseases from the population. Such a process can be expected to affect the distribution of parasite abundance among the host population.

Parasite distributions in a population are known to be highly skewed with a large number of individuals carrying few or no parasites and a few individuals carrying large loads[32–35]. In the study area, other herbivorous mammals such as chital (*Axis axis*) and sambar (*Cervus unicolor*) showed typically skewed distributions[16]. Parasite-free individuals constituted the modal class in these species. There were very few elephants in the parasite-free class (Figure 3). Elephant droppings had a mean parasite propagule density of 19 as compared to 7 of chital and 2.8 of sambar. The shift in the modal class for elephants may not be just an effect of the higher mean value. Two other mammalian hosts in our study area, the omnivorous sloth bear (*Melursus ursinus*) and the carnivorous dhole (*Cuon alpinus*), had parasite-free individuals in spite of having much higher mean parasite densities (130 and 45 respectively).

The positive shift in the modal class of parasite frequency distribution in the elephant population may well be the consequence of selective poaching of tusked males (presumably larger tusked) in this class (parasite resistant). When a negative binomial is fitted to the parasite frequency distributions, the parameter k is relatively low for chital (0.3–0.9 annual range), sambar (0.4), sloth bear (0.4) and dhole (0.28). For elephants the parameter k ranged from 0.5 to 1.83 in different seasons. Higher values of k indicate less aggregated distribution of parasites. Selective poaching for several generations may be one of the reasons for the peculiar distribution of parasites in elephants. A high degree of aggregation is known to stabilize the host–parasite dynamics[34,35]. Populations with less aggregated distributions may become more vulnerable to population depressions from parasitic diseases. Moreover, with the loss of resistance genes from the population, species of parasites which have existed in an endemic state of low virulence for several generations may become a serious health problem. The spate of ivory poaching that swept through Africa and Asia in recent years has to be viewed in this light.

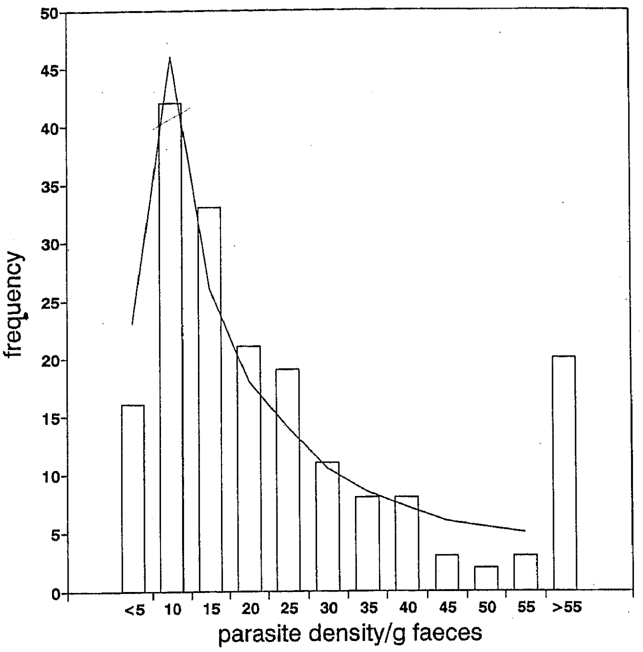

Figure 3. Frequency distribution of faecal parasite propagule densities in the elephant population. The line represents the fitted negative binomial distribution. Parasite distributions are known to be generally positively skewed, with parasite-free individuals forming the modal class. In the case of our study population there is a distinct depression in the parasite-free class. Selective removal of parasite-resistant genes from the population, for several generations may result in such a distribution.

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Current Science

SUBMISSION IN ELECTRONIC FORM

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We expect that electronic submission will result in quicker processing for publication.

Alzheimer's Disease: From Molecular Biology to Therapy. Robert Becker and Ezio Giacobini, eds. Birkhauser, Postfach 133, CH-4010, Basel, Switzerland. 1996. 613pp.

One of the areas of interdisciplinary research that has yet to make a significant advance is the 'Storage and retrieval of information in the brain' or 'mechanism of memory' at the molecular level. The dementias that represent a group of disorders involving loss of memory and other functional aspects of the brain are pertinent in this context. The Alzheimer's dementia is claimed to affect 10 to 15% of human population above the age of 70 in Western countries. Reports on this disease in India are scanty primarily because of the difficulties in the accurate diagnosis and differentiation of this disease from other types of dementia. Increase in the aged population all over the world because of improvements in medical care makes it an urgent need to tackle this disease. The hall-marks of Alzheimer disease are the deposition of a peptide (amyloid peptide of 40 to 42 amino acids) in plaques found in the brain and an abnormal increase in neurofibrillary tangles made of the microtubule protein tau. A marked impairment of cholinergic transmission was noticed in this disease in the beginning stages of research in this area and the approved drugs for amelioration of disease are cholinesterase inhibitors like tacrine which presumably protect the neurotransmitter acetylcholine from destruction. Of late, works from several laboratories have implicated the involvement of several factors such as monoaminergic transmitters, apolipoproteinE, oxidative damage, metal toxicity and presenilin in the pathogenesis of the disease.

This book which is the third in the series of 'Advances in Alzheimer Disease Therapy' provides a number of short articles on current advances in basic and clinical sciences that are relevant in understanding the etiology, pathogenesis, diagnosis, treatment and socio-economic aspects of Alzheimer disease. None of the articles are exhaustive or go in depth into the topic discussed. Most articles give a piece of information that may provide avenues for future thinking or work on the specific topic discussed. Many therapeutic strategies are described; however the use of protease inhibitors to prevent formation of the amyloid beta peptide from its precursor and the use of compounds that would prevent the aggregation and fibril formation of the peptide which are of current interest have not been addressed adequately in the articles. On the whole, this book will provide useful information to those who are already familiar with the varied aspects of Alzheimer's dementia. The last article on the Ronald and Nancy Reagen Research Institute of the Alzheimer's Association emphasizes the gravity of the disease and the need for integrated effort aimed at treatment of the disease.

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Ethical Values for Excellence in Education and Science. J. N. Kapur. Vishwa Prakashan, 4835/24, Ansari Road, Daryaganj, New Delhi 110 002. 1996. Price: Rs 250. 157pp.

Science being the knowledge in pursuit of truth, its very existence demands the premise that knowledge is good and the ethics of responsibility makes scientists individually responsible for evaluating the knowledge that they acquire for transmitting it as may be right and for its ultimate realization as good. Science, thus, demands that ethics be anchored in the very case of reality. Indeed, ethical values must be the starting point and end of all sciences. 'The question of values is the question of the compass according to which we should orient ourselves when we seek our way through life' so reflected Werner Heisenberg.

Albert Einstein spoke on the subject in a very telling way as: 'Our time is distinguished by wonderful achievements in the field of scientific understanding and the technical application of these insights. Who would not be cheered by this? But let us not forget that knowledge and skills alone cannot lead humanity to a happy and dignified life. Humanity has every reason to place the proclaimers of high moral standards and values above the discoverers of objective truths. He must acquire a vivid sense of the beautiful and what is morally good. Otherwise, he with his specialized knowledge will more closely resemble a well-trained dog than a harmoniously developed person.'

Science and society are clearly inseparable. And, so is the congruence amongst values, education and development through science. Education being the most effective institutional means to achieve societal needs and cultural ends by drawing out the best in man, it is the most potent force in inculcating values for propagation of moral and ethical practices. The task of educators is thus to impart a value-based scientific education that concern for mankind always forms the chief interest of all technical endeavours.

Study of ethics is essentially the knowledge to differentiate between the good and bad, right and wrong. It is concerned with discovering the principles that should govern human conduct and with the investigation of normative issues involving value judgements. In a way, ethics join people into societies and yet give them a sense of individuality. Just as fire is essential to produce the best of steel, so are ethics for nurturing the best education and science.

The role of ethics in creating excellence in education, science and society is indeed very fundamental. Its recognition has led to formal courses and degrees and research centres on the subject in several academic institutions in USA and Europe. A new journal on Science and Accountability and a few books based on case studies have appeared. Some Schools of Management and Industrial Engineering have adopted Ethics as a fullfledged subject.

As far as the Indian scenario is concerned, the subject of Science and Accountability has been discussed as a focal theme in the 1994 Indian Science Congress Session. A Society for Scientific Values has been functioning for over a decade: The society held a symposium on Scientific Values and Excellence in Science and published its proceedings. The author of the book under review is a distinguished mathematician, a great teacher and a prolific researcher of renown who has taken keen interest in ethical, moral and scientific values in Indian education, science and society. He has lectured extensively on the subject. Kapur has taken a lot of pains to compile the best of relevant material available in India, including his own lectures and views. The main thesis of the book is that ethical and moral values in Indian society have eroded and continue to do so very rapidly. This erosion has prevented us from achieving levels of excellence in science and education which otherwise we are capable of achieving in all walks of life. The author has compiled available data and dwelt on his personal experiences to support his thesis. Further, he has given suggestions to combat the deadly disease (as he calls it) of Erosion of Ethical and Moral Values (EEMV).

The book consists of three parts. Part A deals with ethical and moral values (EMV) for excellence in science. Part B discusses EMV for excellence in education while part C analyses the subject related to society. Despite the inevitable repetitions and disjointed compilations of various lectures and addresses, the book is well written and is indeed informative and thought-provoking. The contents provide a lot of food for thought for both teachers and the taught, for the researcher at any level, and for the managers of education, science and technology. In summary, the book is a good first attempt to focus the attention of patriotic citizens to a deadly disease which we must tackle before it assumes an epidemic proportion. And, it points out forcefully the need to make ethics as a fundamental subject for students at all levels. It is hoped that this book will inspire more critical discussion, practical initiatives and study materials.

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Neem. N. S. Randhawa and B. S. Parmar, eds. New Age International (P) Ltd. 4835/124, Ansari Road, Daryaganj, New Delhi 110 002. 1996. Price: Rs 500. 332pp.

The multiple efficacy of neem derivatives has attracted the attention of scientists from diverse disciplines, in particular agriculture, public health and medicine. Universal interest is evinced in neem products and their active ingredients. Its utility in plant protection has aroused interest in the fields of agronomy, soil science, entomology, nematology, pathology and chemistry. Viewed in this context, the book under review is a very relevant publication serving to integrate information in the different fields.

Beginning with the taxonomic and vegetative features, reproduction and embryology, wood characters and gum, the book seeks to explain gene resources and breeding potential of neem, in particular seed sources, genetic variation and seed viability storage. Genetic resources of neem and its wild related types are discussed with emphasis on species diversity as well as ethnobotanical aspects relating to local, native and traditional uses and beliefs. Silvicultural aspects including nursery techniques, stand formation and afforestation, growth stabilities, economics of neem plantations and management are highlighted, besides the importance of neem in various agroforestry systems, agrisilviculture, energy plantation and biomass productivity.

The diversity of insect pests of neem and the nature of damage, fungal and bacterial diseases are emphasized and since many pests suffer from natural epizootics, systematic research on insect pathogens hold great promise for IPM programmes. Stress has been made on botanical pest control, also an important component of IPM, involving neem products in pest management and compatibility of biochemicals with biocontrol agents. Chemical aspects of neem involving isoprenoids and non-isoprenoids and their biosynthesis are enumerated with emphasis on limonoids, azadirachtins and melacinins. Steam volatile components of neem seed/oil have also been indicated, besides methods of processing, standardization and product formulation.

An important aspect of neem studies pertains to its nitrification-inhibiting property as well as effects of its application on ammonia volatilization and on the efficacy of nitrogen utilization by crops. Results are also provided in the relative efficacy of urea and neem-cake coated urea in different crops, as well as comparative efficiency of meliacins and nitrapyrin as nitrification retarders in soil. Since neem represents the leading edge of a new wave of botanical bioefficacious products, the need for a standardized evaluation procedure, particularly of the active ingredients in a neem product has been emphasized. In tune with this, different parts of the neem tree, its seed cake, different extracts, oil and gum have been evaluated for their bioactivity against plant parasitic nematodes. Bioactivity against insects, information on their antifeedant activity, insect growth regulating effects, toxicity, effect on fertility and reproduction, oviposition deterrent effect, and effect on natural enemies are adequately stressed. Naturally interest is centred on the mode of action of azadirachtin on these pests, notably physiological mechanisms including endocrines and chemosensory mechanisms.

Neem as an ancient veterinary medicine, livestock production and health and ancient human medicine are documented, besides pharmacological studies for therapeutic potentiality in malaria control. Review has been made of pharmacological studies for therapeutic potential relevant to a number of human diseases, along with commercialization of neem. Since it is a renewable resource of various useful products, opportunities for commercialization are great and useful information is provided relating to this aspect on pesticides and allied agrochemicals, medical application, toiletries and cosmetics, cattle and poultry feed and thermal efficiency of wood for fuel.

In view of the increased consciousness of the role of biopesticides in reducing pollution and their overall efficacy, this superplant neem, has increased potential for multisided utilization. This book is a very useful compendium of diversified information which will go a long way towards kindling further interest in the biological, biochemical and physiological aspects of neem.

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PERSONAL NEWS

A genius called Abdus Salam

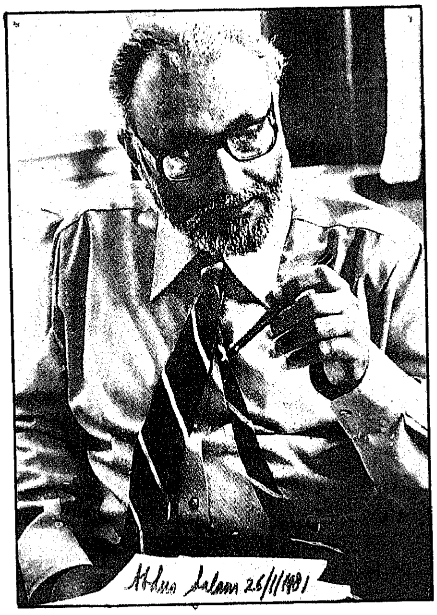
An obituary

Professor Abdus Salam, one of the greatest geniuses of this century, died on 21 November 1996. He was awarded the Nobel Prize in Physics during 1979 for his Electro-Weak Unification theory along with Steven Weinberg and S. L. Glashow. After winning the Nobel Prize he came on a pilgrimage (Ziarat) to Qadian, the holy place for Ahmadias, in January 1981. He visited Guru Nanak Dev University, Amritsar and many other educational and scientific institutes in India. He was conferred Honorary Degrees by many universities and received warm welcome throughout India. When I met him in International Centre for Theoretical Physics, Trieste, Italy during my visit in 1990, he was suffering from a paralytic stroke and moved in a wheel chair. He was as busy as ever correcting the proofs of his paper 'The role of chirality in the origin of life'. He gave me a copy of this paper and confided that he was hoping for a second Nobel Prize in Biology. There are only half a dozen scientists who could score a double in the history of Nobel Prizes. Alas! his dream could not be realized.

Early life and work

Abdus Salam was born in Jhang (India), now a district town in the province of Punjab in the present day Pakistan, on 29 January 1926. He got his early education in Jhang High School and passed his matriculation examination by securing about 90% marks, creating a new record in Panjab University. He repeated this feat in B A and M A examinations also. He joined St. John's College, Cambridge in 1946 and scored a double first class first in both Mathematics and Physics Tripos, a rare feat in the history of Cambridge University. In 1949, he returned to Jhang and got married to his first cousin.

He returned to Cambridge and started off as a research student in experimental physics in 1949. Nevertheless, Salam once put it 'I knew the craft of experimental physics was beyond me – it was the sublime quality of patience – particularly patience with recalcitrant equipment of the Cavendish laboratory that I sadly lacked'. Soon he shifted to Theoretical Physics and started work on Quantum Field Theory with Nicholas Kemmer. He solved an intricate problem, renormalization of meson theories, for his Ph D thesis within five months but the university regulations did not allow him to submit his Ph D thesis before the completion of a three-year period. He was awarded the Smith Prize for the most outstanding predoctoral contribution in 1950 and sent off for six months to the Institute of Advanced Studies in Princeton, USA, where Albert Einstein worked since 1933. He was highly impressed by the greatest genius, the world has ever produced since Newton.



In 1951, Salam returned to Lahore and joined as Professor of Mathematics in Government College and as Head of the Mathematics Department at Panjab University, Lahore. Salam landed in a country where there was not a single person with whom he could discuss his research problems. The nearest physicist with whom Salam could indulge in useful discourse was Homi Bhabha in Bombay and M. N. Saha in Calcutta. After the partition of India, things had changed. Once he travelled to Bombay to meet his friend Pauli, another great Nobel Laureate whom Einstein considered his successor, but on return he was asked to explain as to why had he gone to India without prior permission. He suffered complete isolation in Lahore and could not continue in the kind of environment prevailing in Pakistan if he was to remain in the forefront. During 1953, there were anti-Ahmadia riots in Lahore. Salam returned to Cambridge in 1954 when he was offered a lecturership in physics.

It was in the period 1956–57 that Salam made another astounding contribution. There exists a law known as the law of conservation of parity. It essentially states that if a particular process occurs in nature with a certain probability then its 'mirror image' is also a possible process that occurs with equal probability. It is also called law of left–right symmetry. In 1956, two Chinese-born American physicists, T. D. Lee and C. N. Yang, had proposed a hypothesis that left–right symmetry was violated in certain processes governed by the weak force. C. S. Wu, another great Chinese lady working in USA, experimentally established this hypothesis and was awarded the Nobel Prize for her experiment. Now it was the turn of Salam to prove that violation of left–right symmetry was connected with the zero mass of neutrino. It was the most beautiful mathematical concept, and in establishing it Salam was able to introduce a new type of symmetry that is now called 'chiral' symmetry. According to Salam's two-component neutrino theory, right-handed neutrinos do not exist in Nature. Neutrino always spins in one particular manner instead of the two possibilities expected on the basis of quantum principles. Salam's idea is validated by experiments and is a built-in feature of his Nobel-prize winning electro-weak theory.

In 1957, at the young age of 31, Salam was offered a chair in Theoretical Physics at the Imperial Science College, London, one of the leading scientific institutions in UK. Under his leadership Imperial College came to the forefront of elementary particle physics research and many budding physicists converged to him to seek his guidance. In 1961, Salam and his research students, J. C. Ward and Ne'eman, proposed a new scheme of classification known as Unitary Symmetry independent of M. Gellman. Salam was elected a fellow of the Royal Society in 1959 at the age of 33. He was perhaps the youngest scientist to be elected to this most prestigious society after Ramanujam, the great Indian mathematician whom Salam adored as a young student in Lahore. In 1964, he was awarded the Hughes Medal for his research contributions. Commenting on this award, the issue of *Nature* (December 13, 1964) refers to Salam's work on the two component theory and unitary symmetry as contribution 'in the absolutely highest class'.

Grand Unification Theory

Salam never wavered in his conviction that the unification of the fundamental forces of nature was inevitable. He pursued this goal ceaselessly over the last four decades. Even as a young physicist Salam stuck to this conviction despite the fact that men of the calibre of Dirac and Pauli despaired of the task and discouraged him.

The history of unification ideas is as old as the history of science. Galileo and Newton established that the laws of physics are the same for the earth and the heavenly bodies unlike Aristotle and his followers who considered two different sets of laws operating in the celestial and terrestrial spheres. Faraday and Maxwell unified electricity and magnetism and this unification culminated in a new technological revolution in the world. Einstein was a great dreamer who unified space and time in his theory of relativity and thus the whole edifice of Newtonian physics was shaken. He also proposed a framework for unification of electromagnetism with gravitation. He spent the last four decades of his life searching for a unified theory that would explain everything from elementary particles and their interactions to the overall structure of the universe. But Einstein failed, not because he did not try hard enough, but because the attempt was ahead of its time.

Salam took up cudgels to unify electromagnetism with weak nuclear force. Salam was among the very first few privileged people to realize in the late fifties that the type of symmetry known as gauge symmetry must be the one relevant to all basic forces of nature and to unification. Quantum field theory views all fundamental forces as arising out of the exchange of messenger particles between interacting particles. The range of the force depends upon the mass of the messenger particle. A massless particle gives rise to a force of infinite range. Gauge symmetry further tells us that the gauge particles carry one unit of spin.

The fundamental equations of electromagnetism satisfy a gauge symmetry known as U(1) which requires one massless messenger identified with the photon. The electromagnetic force is left–right symmetric and its range is infinite. The weak force is very different from it. It is a thousand times weaker than the electromagnetic force and its range is incredibly small. Salam proposed that a gauge symmetry SU(2) could be relevant to weak force and its unification. SU(2) requires the existence of three massless messengers. Since the weak force has a very short range, the problem of generating masses of messenger particles was not fully solved until 1967 by Weinberg and Salam, in their Nobel Prize-winning paper. Weinberg and Salam independently of each other took the step of using the Higgs mechanism to predict the masses of two W^+ & W^- particles and Z^o in 1967. It was found that the particles would be about 100 times heavier than the proton. During early 1983, Carlo Rubbia and Van der Meer in CERN, Geneva announced the discovery of W^+, W^- and Z^o messenger particles. Their existence was predicted by the Salam–Weinberg–Glashow model which unified weak and electromagnetic forces. The trio shared the 1979 Nobel Prize in Physics after experiments carried out at CERN, Geneva and SLAC, Stanford, USA established the existence of 'neutral current' processes. However, the climax was reached with the discovery of three gauge messengers which led to vindication of 'Standard Model' proposed by Weinberg and Salam.

In 1972, Salam and Jogesh Pati introduced a bold idea for the unification of electro-weak and strong nuclear forces. Instead of regarding leptons and quarks as fundamentally distinct entities they put them together in a single family or multiplet. Such a scheme then allows a quark to convert to a lepton once in a while. Since a proton is made up of quarks this immediately led to the sensational idea that a proton could decay. The proton lifetime predicted for Grand Unification Theory, is about ten thousand billion billion billion years. Sensitive experiments are being conducted in the USA, the USSR, Japan, India and other places to test the proton decay hypothesis. So far the experiments have failed to detect the proton decay. Salam has also made contributions to 'Super-symmetry' and 'Superstring' theory in his search for the ultimate synthesis of electromagnetic, weak-nuclear, strong-nuclear and gravitational forces.

A prophet of Third World

The creation of International Centre for Theoretical Physics (ICTP) at Trieste, Italy in 1964, under the aegis of UNESCO and IAEA, Vienna, is another great step for unification of Third World scientists. Salam had himself experienced the isolation as a scientist in Panjab University, Lahore.

ICTP has imparted training to almost 50,000 visiting scientists from Third World countries since its inception. The International Centre started with a budget of 350,000 US dollars. During 1987 alone, ICTP welcomed 3,700 physicists and mathematicians to its various activities.

Training colleges and research workshops have been held at ICTP, Trieste on subjects such as physics of materials, alternative energy sources, fusion, reactors, geophysics, biophysics, neurophysics, laser physics, fibre optics, physics of oceans and deserts, soil physics, cloud physics – in addition to high energy physics, condensed matter physics, mathematics and cosmology. Since 1981, research workshops have been organized in developing countries, viz. China, India, Sri Lanka, Bangladesh, Ghana, Kenya, Sudan and Colombia.

Salam is regarded as one of the all-time great scientists, not only because of his scientific achievements but because of the overall impact he has made on the scientific community at present. He believes firmly: 'Scientific thought and its creation is the common and shared heritage of mankind'. However, in science, as in other spheres, this world of ours is divided between the rich and the poor. The richer half – the industrial North with an income of 5 trillion dollars, spends 2% of this – some 100 billion dollars – on non-military science and development research. The remaining half of mankind the poorer South, with one-fifth of this income of around one trillion dollars – spends no more than two billion dollars on science and technology.

Third World Academy of Sciences (TWAS) was founded in November 1983 at Trieste by Salam and a group of forty world-class scientists including nine Nobel laureates from Third World. During 1987–88, he personally visited a number of Third World countries and met various

Heads of States who promised their support. His strong conviction that the current poor status of Science and Technology in the Third World can only be improved substantially by the countries themselves, prompted him to establish the Third World Network of Scientific Organizations (TWNSO) in October, 1983. Salam was elected president and a joint declaration known as 'The Trieste Declaration on Science and Technology as an Instrument of Development in the South' was issued on this occasion. It was Salam's hope that the Network will eventually be an effective force in convincing the governments of the South to at least double their expenditure on Science and Technology and increase the number of scientists and technologists to the level of at least 1000 per million in each country before the end of this century. It is in this connection that he undertook his journey to Bangladesh, India and Pakistan despite his failing health.

If Trieste has become a Mecca for Third World Scientists over the years since its creation in 1964, Abdus Salam, Nobel Laureate and a genius from Punjab, was destined to play the role of a prophet of Third World scientists. May the mercy of Allah be on His servant!

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INTERACTION MEETING IN THE AREA OF CHROMSOMES AND EVOLUTION

The Department of Science and Technology proposes to hold an interaction meeting to generate good, state-of-the-art research proposals for possible funding, in the area of CHROMOSOMES AND EVOLUTION.

Project proposals related to the theme, CHROMOSOMES AND EVOLUTION are invited for consideration. It may include different dimensions such as karyotypic phylogeny, heterochromatin, polymorphism, meiotic mechanisms, non-disjunction, chromosomal rearrangements, supernumerary chromosomes, sex determination, chromosome imprinting, theoretical models, etc.

Those interested in attending this meeting and submitting grant proposals in this area are requested to send a preliminary proposal of their proposed work (5 pages) and a copy of their biodata to:

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DROSOPHILA STOCK CENTRE
DEPARTMENT OF STUDIES IN ZOOLOGY
UNIVERSITY OF MYSORE
MANASAGANGOTRI
MYSORE 570 006

on or before **7 July 1997.**

Applicants should have a permanent position in an academic Institution in India.

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FIRST ANNOUNCEMENT

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Advt. No: 05/97

19 May 1997

Applications are invited for the post of Faculty Fellows (equivalent to Reader/Assistant Professor in a University/IIT) in the Biodiversity Unit of the Centre which is interested in plant, animal and microbial biodiversity. Applicants must have a Ph D degree and two years of post-doctoral experience. They should have a good grasp of quantitative methods in ecology, an interest in evolutionary biology and a genuine fascination for natural history. The post carries a basic pay of Rs 3,700/- in the grade Rs 3700–5700 (the total emoluments being approximately Rs 11,390/- per month).

Applications may be sent to the Co-ordinator at the above address on plain paper along with the detailed CV, copies of the best five publications, a tentative research plan and at least three names of referees.

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All manuscripts should be addressed to the Editor, *Current Science*, P. B. No. 8001, C. V. Raman Avenue, Bangalore 560 080. Submission of an article will be held to imply that it has not been previously published and is not under consideration for publication elsewhere; and further, that if accepted, it will not be published elsewhere. *Three copies of contributions of all categories* are required, with a letter of transmittal giving (i) names and complete addresses of the authors and (ii) title of the contribution and the category in which it is submitted (see below).

Current Science is a multidisciplinary journal and therefore research and review papers of general significance that are written clearly and well organized will be given preference. All papers will be first assessed by a Reviewing Editor. Papers found unsuitable in terms of the overall requirements of the journal will be returned to the authors. The others will be sent for detailed review. *Both solicited and unsolicited material will be reviewed.* Authors of these papers will be notified of acceptance, rejection, or need for revision of the paper. Returned papers cannot be resubmitted.

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Review articles (not exceeding 5000 words) are expected to survey and discuss recent developments in a field. They should be well focused and organized, and avoid a general, 'textbook' style.

Research articles (not exceeding 4000 words) should report research results of fairly major significance. They should include an abstract not exceeding 100 words, introductory paragraph(s), and brief subheads.

Research communications (not exceeding 2000 words) should contain important findings that are novel and of fairly broad interest. They should include a brief abstract and an introductory paragraph. Text should not be broken up under subheads.

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Research news articles are intended to inform nonspecialists about recently published advances or important findings discussed at a meeting. Authors should also send a copy of the paper(s) on which the article is based. Meeting reports should avoid merely listing brief accounts of topics discussed, and must convey to readers the significance of an important advance.

Research accounts articles are intended to be personalized reviews of research from the authors' own laboratory, based on a body of published work. The articles must provide appropriate background to the area in a concise introduction, which should also serve to place the author's work in proper perspective. Articles will normally not exceed 8 to 10 printed pages.

Opinion articles present views on issues related to science and scientific activity. **Commentary** articles should contain expository notes on issues related to science and scientific activity.

Book reviews. Unsolicited reviews will also be considered. Reviews that merely 'list' brief descriptions of the contents cannot be published. Reviews should have 'context' and convey some information about the subject of the book.

Historical commentary and notes inform readers about interesting aspects of personalities or institutions of science or about watershed events in the history/development of science; most are expected to relate to India. Illustrations are welcome. Brief items will also be considered.

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Manuscripts should be typed double-spaced on one side of white bond paper (21 × 28 cm). The pages should be numbered consecutively, starting with the title page and through the text, reference list, tables and figure legends. The **title** should be brief, specific and amenable to indexing. Not more than five **keywords** should be indicated separately; these should be chosen carefully and must not be phrases of several words. **Summary** and **abstract** should not have more than 100 words and should convey the main point of the paper, outline the results and conclusions, and explain the significance of the results.

Text. All papers should have a brief introduction. The text should be intelligible to readers in different disciplines and technical terms should be defined. Tables and figures should be referred to in numerical order. All **symbols** and **abbreviations** must be defined, and used only when absolutely necessary. Superscripts and subscripts and ambiguous characters should be clearly indicated. **Units of measure** should be metric or, preferably, SI. Methods should, as far as possible, be described briefly in appropriate table and figure legends.

Figures. In the case of line drawings one set of originals (without any lettering) is sufficient, with two copies containing lettering. In the case of photographs good prints are required with each copy of the manuscript; photocopies are not acceptable. Line drawings should be roughly twice the final printed size. The correct orientation should be indicated if not clear.

Photomicrographs and other photographs that require it must have a scale bar, which should be defined clearly in the legend. Primary data should be submitted as far as possible (e.g. actual photographs of electrophoretic gels rather than idealized diagrams).

References should be numbered in the order in which they appear, first through the text and then through table and figure legends. The following are examples of ways of writing references.

1. Mukundan, T. and Kishore, K., *Curr. Sci.*, 1991, **60**, 355–362.
2. Constantine, G., in *Biology of Bats* (ed. Wimsatt, W. A.), Academic Press, New York, 1970, vol. 1, pp. 319–322.

Acknowledgements should be brief. Footnotes are not allowed except to identify the corresponding author if not the first.

Cover photographs. Good photographs (colour or black and white) that pertain to a submitted paper will be considered for use on the cover. Good *prints* and a legend should be submitted with the manuscript. In the case of a colour picture, a transparency will be required for printing if accepted.

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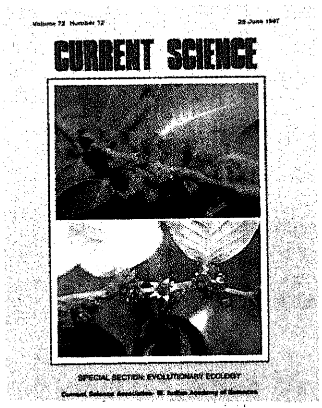


COVER. In individual *Bridelia retusa* trees, male (top) and female (bottom) flowering phases alternate several times throughout the flowering season. This system of sex expression is called temporal dioecy. See page 940. (Photo: Renee M. Borges).

Indexed in CURRENT CONTENTS/GEOBASE/CHEMICAL ABSTRACTS

The editors thank Prof. Raghavendra Gadagkar, Centre for Ecological Sciences, Indian Institute of Science, Bangalore for agreeing to be Guest Editor for the Special Section.

In this issue

Medical research in India: In need of intensive care

The availability of the *Science Citation Index (SCI)* databases has spawned an enormous variety of studies of science and scientists. It is now possible to generate all manner of data on publishing scientists, journals and disciplines. In this issue (**page 912**), Arunachalam asks the question 'How relevant is medical research done in India?' To answer this, the author turns to the *Medline* database and not the *SCI*, because during the period under study (1987–1994), the latter covered only a *single* Indian medical journal – a commentary both on the *SCI* and the perceived standard of most of our medical (and indeed all science) journals. Can searches for 'Indian' papers yield correct results? Arunachalam describes the inclusion of all possible Indian cities in 'address fields' for computer searches; a process that provides him a respectable number of publications for analysis. He then proceeds to draw many important and provocative conclusions. In particular, as compared to agriculture, medical research in India appears to be limping and off target. This is hardly surprising since biomedical research has never been a major thrust in our medical institutions, with one or two important exceptions. The jealous guarding of 'turf' in medical colleges and higher institutions by medical degree holders has ensured that basic science, an important pre-requisite for creating a research ambience, is largely excluded from the teaching of medicine. Pharmacology, microbiology, immunology and genetics are the step-children in medical course lists, leading to the production of a large number of clinicians with only a limited appreciation of the capabilities of modern science in biomedical research. The spatial separation of research institutions and hospitals hinders fruitful interactions between researchers and clinicians. This gulf has hindered the growth of modern biology in India directed towards the purposeful attack on problems of local importance. Few lessons have been learnt from the burgeoning biomedical research enterprise in the West. Ironically, the Indian Council of Medical Research (ICMR) is chronically underfunded, struggling to maintain a laboratory network which is hardly suited to the demands of modern science. Fortunately, both CSIR and DBT appear to be moving towards shoring up biomedical research, although an infusion of funds is hardly likely to bring about the attitudinal changes that appear necessary.

While Arunachalam's critique may hardly be music to the ears of the medical establishment, there is no doubt that it must be heard. In a perceptive commentary, Valiathan (**page 911**) points out that the 'poor correlation between major health problems and the preferences of investigators has reasons which go deeper into the history and evolution of medicine in India'. He concludes that 'India welcomed western medicine in the nineteenth century and quickly learnt to use its tools and methods without bothering to learn how to make the tools and methods. This failure, like a birth defect, became a handicap and ensured that successive waves of tools and methods from the West, and not societal needs, determined the medical agenda in India. In many ways, this is reminiscent of Indian scientific research in general: it remained aloof from the people and failed to power our socio-economic development which continues to progress by infusions of foreign knowhow.' While any critical analysis of medical research (or indeed of any other area) will be bound to attract comment, in an environment where criticism is hardly ever construed as constructive, it is clear that with the increasing availability of bibliometric tools this is unlikely to be an isolated exercise.

P. Balaram

Evolutionary ecology

Spectacular advances in our understanding of the molecular mechanisms of life processes have legitimately led to a feeling that we now understand the logic of life. It turns out, however, that the molecular logic of life does not always enlighten us sufficiently about how whole organisms behave. Evolutionary ecology is emerging as a new way of understanding the logic of life at various levels of biological organization. This is an area in which Indian scientists have a particular advantage because of our rich biological diversity and because there is relatively less need for expensive and sophisticated technological inputs for its pursuit. This issue of *Current Science* carries a special section (**pages 923–956**) on evolutionary ecology, based on papers presented at a symposium held during the 62nd annual meeting of the Indian Academy of Sciences at Jodhpur in November 1996. The six papers in this section – chosen to represent diversity, plant and animal, vertebrate and invertebrate, theoretical and empirical – provide a glimpse of the growing attention that evolutionary ecology is receiving from biologists around the country.

The love life of frogs is a favourite topic of study for ethologists. The use of loud and noisy calls by the frogs adds to the fascination of the subject. Perhaps because of the nearly exclusive contribution to the field by male scientists, frog courtship has largely been considered to be a male-dominated affair. The calls given by male frogs to attract females, have been subjected to very detailed analyses.

Long hours of observations in the marshes of North Eastern India have now begun to show that female frogs play a no less decisive role. Their calls may be feeble but appear to be no less clear in their meaning. On **page 923** Debjani Roy provides a lively description of frog courtship, based on 358 days (nights, really) of observations.

A bat will swoop down in complete darkness and make no mistake in picking up a frog before you can blink your eyelids. Animals often perform such feats that challenge our imagination. Systematic phenomenological investigations often make animal behaviour less mysterious but no less impressive. Persistent and meticulous investigations of the question of how bats detect their prey demonstrate that bats employ contrasting methods of catching their prey in land and in water. To catch frogs on land, bats use a passive 'listening in' method but to catch a frog in water they use an active 'echolocation' method. On **page 928**, G. Marimuthu shows why stationary prey insures life while moving prey ensures death in the world of bats.

Cooperation and conflict are inseparable components of biological organization. The theoretical understanding and empirical demonstration of substantial conflicts between parents and their offspring have been major achievements of modern evolutionary ecology. We have now begun to realize that the same rules apply even to the apparently 'passive' plants and that the various hormones produced during plant growth and development can be interpreted as modulators of parent–offspring conflict and cooperation. Arguing that though they can neither sing nor dance, plants indulge in sibling rivalry, fratricide, and kin cooperation as intensely as animals do, on **page 932**, R. Uma Shaanker and K. N. Ganeshaiah continue their crusade for the cause of plant ethology and sociobiology.

While male and female flowers are produced on different individuals in dioecious plant species such as the *Papaya* for e.g., most plants are hermaphrodites – with anthers and stigma in the same flowers or at least with male and female flowers on the same tree. Nature seems to have evolved a bewildering array of mechanisms to help avoid self-fertilization in such monoecious species. Temporal dioecy or the separation of male and female flower production in time is one such strategy. Studying populations of *Bridelia retusa*, a member of the family Euphorbiaceae in the Bhimashanker wildlife sanctuary near Pune, Renee M. Borges and her students find that this species has the habit of changing sex (of its flowers) repeatedly between male to female and vice versa. What is the evolutionary advantage of such varying gender? See **page 940**.

It is reasonable to assume that the currently observed phenotypic variation in any organism is the combined effect of its ancestry, random genetic drift and natural selection (past and present). But what are the relative contributions of these various factors? The answer usually is anybody's guess, but with two exceptions. R. E. Lenski and coworkers have used the bacterium *Escherichia coli* while Amitabh Joshi and co-workers have used the fruit fly *Drosophila melanogaster* to make an explicit attempt to apportion the observed phenotypic variability between the above mentioned factors. Being a sexually reproducing organism, *D. melanogaster* exhibits, compared to *E. coli*, a smaller effect of history and a higher rate of adaptive evolution – differences that A. Joshi (see **page 944**) rightly attributes to the role of genetic recombination.

Competitive selfishness is the corner-stone of natural selection but we see many instances of the evolution of cooperation and even of altruism in the animal kingdom. Ants, bees and wasps are prime examples of this phenomenon. Understanding the evolutionary forces that promote the evolution of altruism has been a major obsession with evolutionary biologists in recent times. The direction of evolution from the selfish, solitary state to the altruistic, social condition has usually been thought to be irreversible. Recent studies on honey bees, halictine bees and ponerine ants lead to the hypothesis that nature has indeed rewound the tape. Understanding the emergence of selfishness in a group of altruists, promises to become a whole new field of inquiry (see **page 950**).

Raghavendra Gadagkar

CURRENT SCIENCE

Volume 72 Number 12 25 June 1997

CORRESPONDENCE

Massacre of mahseer fish

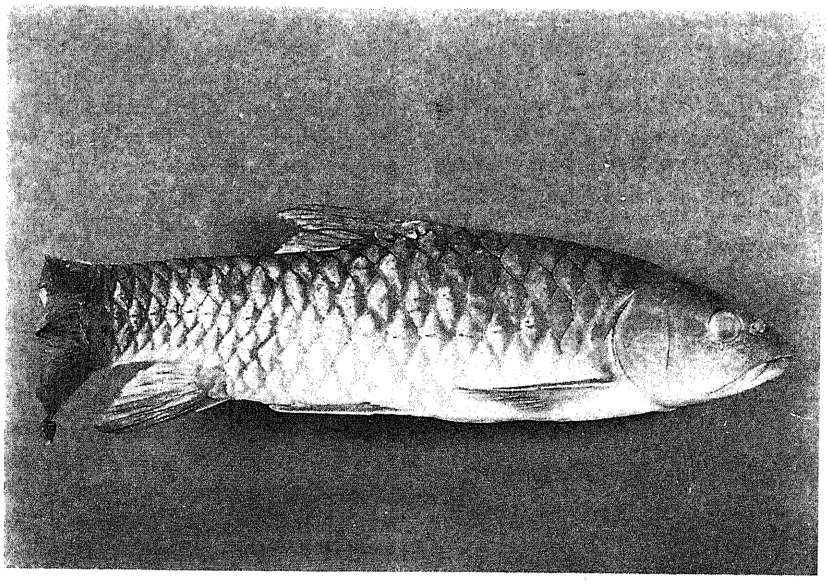
On 26 May 1996, tragedy struck the congregation of mahseer fishes at Shishila near Sulya, a small village in Chikmagalur district, Karnataka. The entire lot of fish, all mahseers, zealously guarded and protected by the local villagers in the river Kapila flowing by the side of Sheshileshwara temple was poisoned by fishermen with severe toxic compounds, killed mercilessly and thus made unfit for any purpose. The reason was that being a protected place, a temple sanctuary, where fishing has been strictly prohibited from 1938, the villagers reprimanded four fishermen for fishing illegally in the night. The fishermen in turn, wrought destruction on the fishes.

It had been my good fortune to visit the same spot in April 1996 in connection with my project on mahseer fishes and I had observed several of them, adult ones swimming merrily.

In many river stretches where a temple or a religious site is located, mahseer fishes are in congregation. They come to the surface and are fed with puffed rice by pilgrims. The rivers have been stocked with fries and fingerlings by the Karnataka State Fishery department, having obtained them from the fish farm of the Tata Electric Company at Lonavala where artificial hatching and breeding of mahseer fishes are in vogue.

Such river sanctuaries for mahseers exist on the Thunga river at Chiplagudde near Teertahalli (Shimoga district), Sringeri and on the Cauvery at Ramanathapura (Hassan district). Almost all the fishes are the common *Tor khudree* Sykes, though the endangered *Tor mussullah* Sykes is also reported to be present.

This tragedy highlights the socio-economic compulsions of the end users who are fishermen and villagers, whose livelihood and sustenance should be kept in view. A regulated exploitation may serve both ends of compensation and conservation. This matter was extensively reported by Dinesh Patvardhan in the Kannada magazine *Sudha* in the 30 June 1996 issue.



Lateral view of *Tor khudree* from Shishila (standard length = 350).

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CORRESPONDENCE

X-ray crystallography in India

Having been associated with S. Ramaseshan as a research scholar in the Physics Department, Indian Institute of Science, during the period 1958–62, it was with considerable pleasure that I read his absorbing article 'Dorothy Hodgkin and the Indian Connection' (*Curr. Sci.*, 1997, **72**, 457–463). This is one of the introductory articles of the *Collected Works of Dorothy Crowfoot Hodgkin* published by the Indian Academy of Sciences, wherein he describes the influence Dorothy had on the growth of X-ray crystallography in India. After discussing at length the contributions of Indian scientists at Oxford, and in India after their return, Ramaseshan talks briefly about other crystallography groups in India. In this connection I would like to mention my own little contribution to inorganic crystallography.

Around 1964, the Inorganic and Physical Chemistry Department, IISc., in a far-sighted move, decided to recruit a faculty member in X-ray crystallography to train students of chemistry in chemical crystallography and initiate research in this field. I realized even at that time that the future growth of crystallography would take place in departments of chemistry, where new molecules are synthesized, and that is where I wanted to be. That is how I happened to join the Department as a lecturer in the summer of 1965. I feel that I can claim with justifiable pride that I was the first to initiate teaching and research in the field of X-ray crystallography in a chemistry department in India, all the work until then being done in physics departments. As described vividly by Ramaseshan, 'Venkatesan's complete conversion to organic chemistry took place through his association with Jack Dunitz in Zurich and Dorothy in Oxford.' He joined the Organic Chemistry Department, IISc., in 1971 to establish 'one of the better laboratories in organic and drug chemistry'.

To conclude, I believe that both Venkatesan and I can look back with satisfaction on our diverse contributions to structural organic and inorganic chemistry and on pooling our efforts to promote crystallography among chemists in India.

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Agriculture in floating fields on Loktak Lake, Manipur

In Manipur, agriculture is not confined only to land, but also extends to the floating fields on the lake (Figure 1). It appears that the people around the Loktak lake, particularly in the Thanga village, because of limited land space for cultivation, had earned experience, through trial and error, of the use of water environment in the cultivation of plants. For this they first collect the floated mass of water grass at a particular place in the Loktak lake and spread it approximately to an area of about 0.62 acre or more. The thickness of such a mass of floated grass varies from 1 m to 2 m. They burn the long grass that rose above the water. Then the sprouted young sots are again cut with a knife to allow decay with the ash to form a rich organic soil-like field.

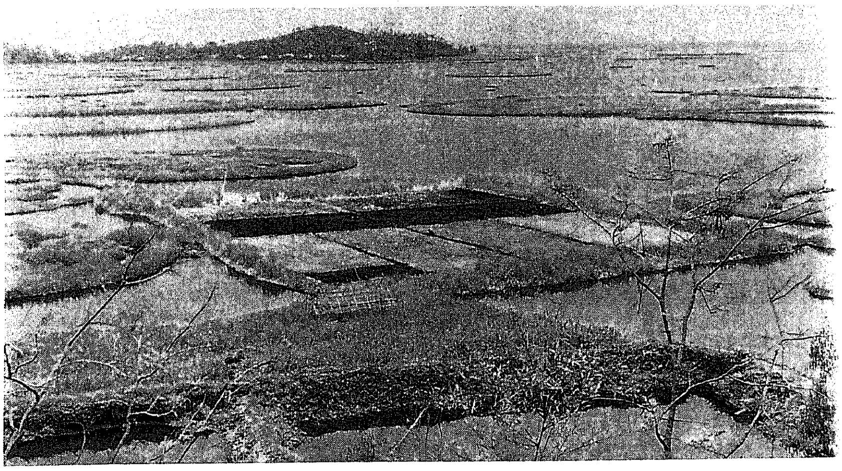

Figure 1. Floating fields.

Now the field is ready for the transplantation of young paddy plants that have been grown in the land nursery field. Such floating fields can be used effectively for three years continuously after which the field becomes thin and loses fertility. After three years, some people upturn the floating field and the roots of the water-grass are allowed to decay to enhance the fertility of the field or a new field is prepared. Now double cropping of paddy is also done, and April is the season for the plantation of the first crop. The yield per acre is said to be almost equal to that of the land agricultural field.

There are both advantages and disadvantages of cultivation in such floating fields. As the field is being floated, there will be no problem of destroying the crop by water, rather cultivation can be done one after another throughout the year. However rodents, particularly the rats, destroy the crop very easily and birds too eat up the crops in the fields that are far away from the permanent village at the foot-hills. Of course, people construct floating huts by their floating fields to watch the crop and to protect it from rodents and birds. Yet they face problems for the protection of the crop. Transportation of the crop from the floating fields to their village is done with dug-out canoes.

It seems to be the first instance where a floating mass of grass on the lake has been used for rice cultivation. If the laborious technology in current use is changed with new improved methods that may be developed by the agricultural scientist, we may use the many presently uncultivable inundated places and may help in bringing about a better economy. Since the major part of the earth's surface is covered by water, the extension of using the water surface for agricultural work will be of global interest.

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Modelling biological systems*

At the end of his life, the master sleuth Sherlock Holmes said, 'Of late I have been tempted to look into the problems furnished by Nature, rather than those more superficial ones for which our artificial state of society is responsible' (Final Problem). He realized that no riddle can be more challenging than a natural one. Because he was well aware of the complementary roles of facts, hypotheses and theory in solving riddles, he said 'It is of the highest importance in the art of detection to be able to recognize out of a number of facts, which are incidental and which vital', and that 'It is a capital mistake to theorize before one has data'. He also knew that it is possible for people to 'come into those conjectures where the most logical minds may be at fault; each may form its own hypothesis upon the present evidence, and yours is as likely to be correct as mine'. A complete solution involves not only the collection and understanding of data, but also the use of logical and mathematical reasoning to draw inferences in a self-consistent fashion. And isn't that what a scientist aims to do?

Why modelling?

Though formalization or modelling has been a common approach to problem-solving in all branches of knowledge, its use in biology has not been all pervasive. Modelling biological systems are both complex and challenging. This is mainly because they contain extensive coupling, high degree of redundancy and widespread nonlinear interactions moulded by millions of years of evolution. This has led modern biology to become largely an experimental science, continuously striving to explain mechanisms in detail. The explosion of physio-chemical information at the molecular level of biological processes/systems necessitates an effort to organize all data into models which would represent the processes/mechanisms/structures being studied in generalized abstract forms (schemes). Mathematics is used to model the schemes and the predictions of the models are checked with existing and new experimental data. New experimental data may support, enhance (by adding more features), alter, or even falsify the model. There are celebrated examples of pooling of facts into a general framework of understanding (theory) in the concept of operon; or, have detailed description of the processes in the form of compact equations (laws) in Mendel's Laws of genetics. It is probably now all too common to be able to appreciate the role of enzyme kinetics theory in explaining the phenomenon of allostery, feedback regulation, molecular interactions such as DNA melting or promoter search. In all the cases the 'models' help in categorizing a large pool of experimental facts into a coherent and logical understanding which then provide direction to further experimentation. Modelling offers a powerful analytical research tool to a biologist. And that is why wise men have said 'Theory without facts is fantasy, and facts without theory is chaos'.

The complexity of biological systems makes interdisciplinary involvement essential. Increasing use of mathematics or theoretical studies is inevitable as biology becomes more quantitative. There is another compelling reason to study biological systems from the theoretical point of view. Living systems are perfect examples of complex systems, and the interest in understanding complexity at a purely abstract level allows the theorist to gain insight from the new and exciting natural complex processes, thus enhancing the conceptual knowledge base. This level of enquiry may not seem biologically relevant at present, but it, in turn, can be of much relevance in future experimentation or technology. But for this to happen most fruitfully, the theoretician needs to understand the phenomenon being modelled in great detail, and the biologist needs to look for the underlying generalizations in their sphere of enquiry and appreciate the validity of the assumptions in theoretical methods. Both have to be aware of the conceptual developments in other fields.

Indian scenario

The present scenario in theoretical studies in biology in our country is far from adequate. Apart from the widely known and significant contribution in the area of molecular structure and conformations (*a la* Ramachandran Map), theoretical studies in other areas are scarce. Given the water-tight nature of our academic courses, no common language has developed which can allow a common minimum interest/dialogue to evolve among the students in different branches of science. In the course of research activity also, workers in each discipline do not *usually* choose interdisciplinary approaches. No wonder that one hardly comes across any mention of this area in any major society meetings in this country which has a large students' and workers' base. Nevertheless the need has been felt and there has been some activity through small, occasional meetings (e.g. Mahabaleswar seminars, discussion meetings of the science academies, etc.) addressing questions in the area of modelling and theory in biology. These have evoked considerable interest among students and researchers, but most of it has dissipated due to lack of continuing interaction and follow-up.

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*Report on the National Seminar on 'Modelling Biological Systems: Concepts and Applications' held at the Centre for Cellular and Molecular Biology, Hyderabad during January 8–9, 1997.

A recent meeting

A two-day meeting on 'Modelling biological systems: concepts and applications' was held at the Centre for Cellular & Molecular Biology (CCMB) – a constituent laboratory under the Council for Scientific & Industrial Research (CSIR) at Hyderabad during January 8 and 9, 1997 with the aim to collect like-minded workers in one place and assess the state of the art as exists in the country. Though the major thrust of research in CCMB is in experimental biology, it has a small but dedicated group engaged in varied aspects of theoretical studies of living systems such as, statistical analyses of molecular sequences, modelling structures of peptides and proteins, mathematical modelling of biological processes and phylogenetic analyses. Thus it provided a convenient and useful backdrop for holding a meeting of this sort. About thirty people gathered from all over the country at CCMB to deliberate, discuss and listen to what the other had to say. Expertise ranged from molecular biology to statistics, mathematics to chemistry and physics to ecology. Each person looked at the 'elephant' from his/her angle, and all realized how wide the gap is between theory and 'reality' – a 'reality' that changes its nature with new experiments! The scientific programme consisted of two special talks and sessions on five themes spanning all levels of biological organization – Genetics and Evolution, Molecular Structure and Organization, Pattern Formation, Modelling Disease, and Complex Systems. There was also a poster session with work from physical sciences and biology.

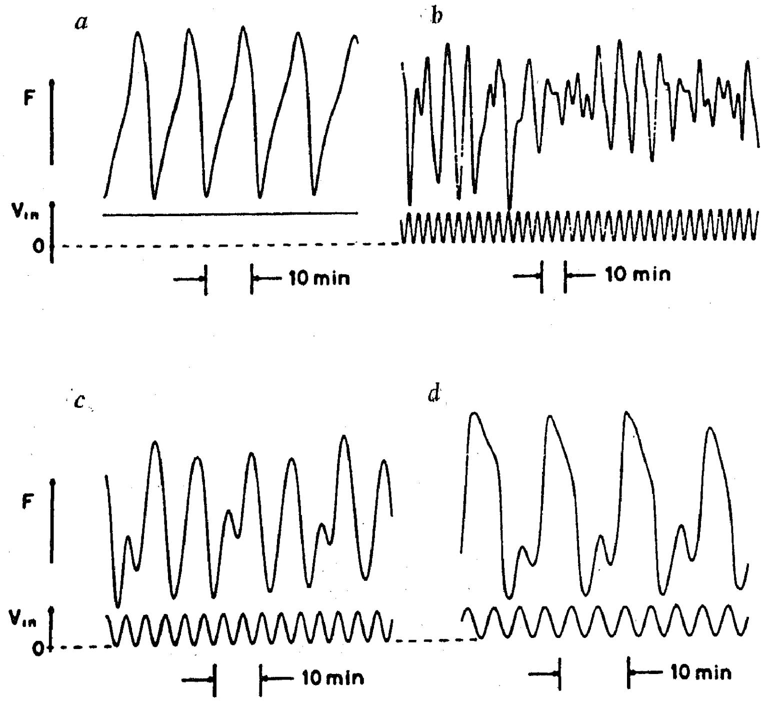

Figure 1. Simple and complex oscillations in a biochemical pathway (NADH fluorescence (upper trace) in yeast glycolysis) under different conditions of input glucose flux (lower trace). ***a***, Oscillations at constant input flow; ***b***, aperiodic; ***c***, 1:5 entrainment; ***d***, 1:3 entrainment. (From Markus *et al.*, *FEBS Lett.*, 1984, **172**, 235–238).

Theory in biology

The special talks were ideal examples of the complementary ways of using theory to model biological systems. One of the most interesting phenomena in living systems is the ubiquitous periodicity observed in all time scales in life – from the short period heart beats to long period cycles of hibernation, including the daily or circadian rhythm. It stands out against the deep-rooted 'homeostatic' view of life. Such oscillatory dynamics are also close to the heart of theorists since they represent some sort of instability in the functioning of the system. Abstract models of many of these rhythmic phenomena were proposed earlier till the cellular, molecular and genetic bases were identified. Albert Goldbeter (Free University at Brussels, Belgium), in his talk on 'Biochemical oscillations and cellular rhythms: From experiments to theoretical models', discussed mathematical models of some well-known oscillatory processes in biochemistry (e.g. the glycolytic oscillations, cell division cycles, calcium oscillations and circadian rhythms in *Drosophila*) based on experimental observations. It identified those molecular processes which act as positive feedback and forcing mechanisms which give rise to the instability and consequent oscillatory behaviour. The theoretical analyses also clarified certain difficult experimental results. On the whole, it helped towards better description of the system behaviour and directed future experimentations. It also brought to attention the conditions where simple periodic behaviour in physiological functions could transform into complex oscillations, including bursting and chaos, a phenotype observed in many experimental situations or mutants (e.g. complex glycolytic oscillations, aperiodic mutant of slime molds).

In contrast to this approach of modelling different periodic processes using similar kinetic schemes, Lee Segel of the Weizmann Institute, Rehovot, Israel, in his talk on 'Immunity and Autoimmunity: What can theory tell us?', used three different theoretical approaches to study the complex biology of the immune system. He analysed a system of ordinary differential equations to model the classical method of immunotherapy for allergy; a reverse engineering approach to model many experimental observations concerning the T-cell vaccination approach to counter autoimmune diseases; and, simply using concepts of feedback without any equations, provided a possible framework of the overall operation of the immune system. In all of these, the actual system under study was clearly defined in molecular and cellular terms before modelling and yet simplifications were made for the analyses to yield fruitful results based on which realistic conclusions could be made. The ideas generated through theoretical studies were verified by appropriate experiments. His treatment of theory in understanding biological systems left a deep impression on everybody irrespective of their scientific background and set the mood of the meeting. These talks were appreciated by experimental biologists and theorists alike.

Problems furnished by nature

The session on Genetics and Evolution addressed some very relevant and important questions in the study of evolution and maintenance of genetic diversity in human populations. Evolutionary theory, with its rigorous logical and mathematical framework, has been an area of interest to experimentalists in plant/animal breeding, physical anthropology and population genetics. Theoretical studies (Partha P. Majumdar, Indian Statistical Institute (ISI), Calcutta) have been able to explain the types of genetic differentiation that can be caused by subdivision of populations based on specific geographical or social factors. Such findings can be very useful in areas related to prevalence of genetic diseases in communities. Theoretical methods have commonly been used in trying to find the origins of and the relationships among different groups of human populations (phylogenetic analysis), and every method has certain underlying assumptions threaded into it. Much of the theoretical interpretation depends on the nature and mode of data collection, and the models of genetic distances among the populations. Thus it is a challenge in evolutionary studies to find the most reliable description of the past based on present data. The problems and methods were discussed in this session and a map-representational approach was proposed for the analysis of affinities among human populations all over the world (Chandrika B-Rao, CCMB, Hyderabad).

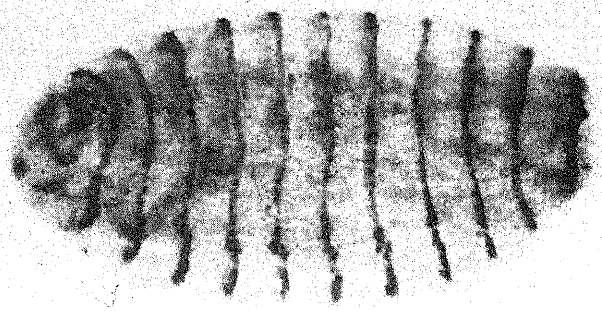

Figure 2. Spatial pattern of gene expression during embryogenesis. Expression pattern of a reporter gene construct in the anterior compartment of every segment in a stage 12 Drosophila embryo (From L. S. Shashidhara, CCMB).

Most interestingly, 'chance' plays a profound role in genetics and evolution. Thus the genetic algorithm (GA) framework based on 'coin tossing experiments' to model evolutionary search is used in many theoretical studies for explaining adaptation and evolution of genetically diverse populations. Capability of exhibiting phenotypic plasticity even in a fixed environment was modelled using the GA method (Narayan Behera, Jawaharlal Nehru Centre for Advanced Scientific Research (JNCASR), Bangalore) and it was shown that under certain conditions it was possible to speed up the rate of evolution (adaptive evolution) through trans-gene regulation of 'potentially' plastic alleles.

The session on Molecular Structure and Organization aimed at identifying the concepts underlying the structure, organization and function of biological molecules, organelles and other organized structures inside the cells. In addition to thermodynamic and other physico-chemical considerations, this session also looked at structural and experimental models which were firmly based on molecular biology and biophysical studies. Description of cellular organization, starting from cellular signalling to the structure of the molecules of life, involves similar concepts. The physico-chemical principles for designing structural motifs (bends or turns in a local scale) in proteins, which are also the backbones of active sites and enzyme actions in cellular processes, was elaborated (R. Nagaraj, CCMB, Hyderabad). This has far-reaching applications in protein engineering and drug design. It is known that proper folding of large molecules (proteins) is absolutely important for their proper functioning, and misfolded enzymes have been the source of some metabolic diseases. In modelling the mechanisms of protein folding, one hypothesis (T. A. Thanaraj, European Bioinformatics Institute, Cambridge, UK) has looked for the influence of the kinetics of translation itself on cotranslational folding events. Results of statistical analysis of a large number of proteins from prokaryotes show that higher order structural organizations are coded by translationally slow regions on the corresponding messenger RNA (mRNA). There is also a correlation between the codon usage in mRNA and topological features (alpha helices and beta strands) on the encoded protein. The next level of structural complexity involves organizations like membranes and multi-protein assemblies performing cellular functions. A conceptual framework was advanced (Biman Bagchi, Indian Institute of Science (IISc), Bangalore) where the different morphologies (helical and flat) assumed by amphiphilic monolayers and bilayers were shown to be driven by chirality. This theory is based on thermodynamic and molecular parameters such as pair potential, group size, and the orientation and distance between the groups.

More complex jobs in the cells are performed by higher order structures made by multi-protein assemblies. The motor apparatus controlling the movement of tiny tail-like organs (flagella) propelling unicellular organisms in swimming is a typical example of such a specialized function. Modelling this process (Shahid Khan, Albert Einstein School of Medicine, New York, USA) needed, first, the complete description of the molecular architecture of the flagellar motor assembly in terms of molecular biology and biophysics. But the characterization of the stochastic properties of the rotation and switching is modelled in terms of mechanics and mathematics. These types of structural models and quantitative analyses can describe the mechanism of action of many molecular motors in living systems.

Emergence of ordered structures or patterns is one of the most interesting and actively pursued areas in theoretical and experimental biology today. Symmetry, breaking of symmetry, self-regulation and duplication of patterns, diffusive instability, etc. are attractive images to theoreticians. These are also shown to occur in many experimental situations such as, during embryogenesis, chemotaxis of cells, in excitable tissues like heart, neural conduction, and even during hallucinations. The session on Pattern Formation consisted of lectures on recent theoretical research in physics and mathematics of spatially-extended systems. They considered abstract mechanistic models that can give rise to both stationary and nonstationary patterns such as, stripes, hexagons, spirals and waves which are found in diverse biological processes. The theoreticians, on the other hand, got exposed to the recent genetic and molecular data and currently understood mechanisms of animal development. It is known for a long time that chemical reactions with autocatalytic and inhibitory steps coupled with diffusion mechanisms can give rise to a wide variety of spatiotemporal patterns. Some of them (e.g. spots) can even grow, replicate, or die (V. Ravikumar, National Chemical Laboratory, Pune) which could be of interest in biology. Such reaction-diffusion models can be built with limited or even inaccurate system information. Strategies to control the nature and properties of these patterns can be used to obtain desirable features such as, altering and arresting the growth of replication. There are some powerful models existing in the literature for spatially-extended systems (e.g. the Ginzburg–Landau and the Fitzhugh–Nagumo equations) which have been used to describe phenomena such as aggregation and propagation of nerve action potentials. Further analysis of these equations showed (Rahul Pandit, IISc., Bangalore) possibilities of encountering spatiotemporally chaotic states under some conditions. The different ways of characterizing the transitions between these states were discussed from a theoretical point of view. Though the concept and experimental verification of chaotic states in patterns have been considered in chemical and biochemical processes, it still has not caught the fancy of the developmental biologists. The possibility of finding general molecular pathways in animal development, deviations from which can give rise to abnormalities, may have some relevance in such ideas.

An overview of the recent advancements in the area of animal development (Sudipto Roy, National Centre for Biological Sciences, Bangalore) was an eye-opener for the theorists of the levels of complexity that biologists have to work through to get even a glimpse of any general mechanisms operating at the molecular level during the varied time scales of development. A world-wide effort of many workers using a plethora of molecular and cellular techniques has now shown that a class of proteins (hedgehog family) could be considered a good candidate for the organizing activities and positional signalling in the fruit fly *Drosophila* and other vertebrates. There are also evidences where mutations in the human hedgehog gene lead to congenital abnormalities and neoplasia. The complexity of molecular processes involved and the rate at which experimental information is accumulating (and changing) from studies on a variety of organisms, makes it a very active area of research with continuous overhauling of ideas. Historically, developmental biologists have had an affinity towards theoretical modelling based on experimental results, yet keeping track of the present explosion of information and understanding the intricacies of the systems are not easy tasks for the modellers.

One of the major reasons for studying biological systems is to be able to understand why and how the normal behaviour is altered and disease conditions appear, so that one can find out ways of better treatments. Theoretical advancements have had various uses – by proving the basis of medical technology or even simply predicting conditions for the altered state of the normal behaviour. The session on Modelling Disease concentrated on modelling the normal dynamics (behaviour) and then attempted to identify the conditions/parameters/interactions that gave rise to the altered or abnormal dynamics. Disease, in some sense, can be thought of as emergence of disorder in an ordered state through changes in specificity or strengths in metabolic reactions or nonfunctional genes; or, due to active interaction with pathogenic agents, such as infectious agents or toxic chemicals. Theoretical methodologies considered here ranged from neural network modelling to the geometrical approach to reaction-diffusion equations with spatially variable cross-diffusion. While considering interaction of pathogens with the immune system (Prabhakar Vaidya, CSIR Centre for Mathematical Modelling & Computer Sciences, Bangalore) interesting conclusions about the system behaviour were obtained, using nonlinear dynamics and geometrical approach with graphs, from very simple assumptions which can be easily understood by non-mathematicians. Though having analytical solutions of equations is quite difficult considering the high degree of nonlinearity involved in biological processes, it still is the only way to obtain a global picture of the system behaviour. In search of general mechanisms, pure analysis of a reaction-diffusion model (Joydev Chattopadhyay, ISI, Calcutta) showed that negative cross-diffusion can play a crucial role in generating stable dissipative structures, and spatially variable diffusion and loss of cell-to-cell contact can lead to disordered growth (tumours). Such scenarios are quite common in cell-to-cell signalling through diffusive molecules and tissue growth during morphogenesis.

Neural network models have been used commonly to describe the neurobiological processes. A network with biologically relevant features such as, synaptic specificity, delays and plasticity, were used (Chandan Dasgupta, IISc., Bangalore) for modelling the phenomenon of 'kindling' of focal epilepsy. The model argued that the network accumulates a large number of excitatory synaptic connections through a Hebbian learning mechanism which gives rise to 'kindling'. It also predicted new experiments for validating the proposed mechanism which also sheds light on the nature of memory storage in the brain. Along with obvious orientation towards applications in therapeutics and diagnosis, these theoretical studies also provide enhancement of conceptual knowledge.

The final session of the seminar was on Complex Systems and covered studies ranging from molecules to populations, including models of behavioural functions. It is worth remembering here that even the simplest of the life processes on earth has evolved through millions of years of continuous manipulation of different forces; and what one sees now represents one snapshot of its spatiotemporal existence during its on-going evolutionary history. This has led to complexity at every level including organization of information in biological molecules. The world-wide programmes of the genome analyses of some model organisms (e.g., bacteria, yeast, humans) aim at deciphering the huge information coded in the large base sequences. Capturing distinctive features of such large nucleic acid sequences is one of the most active research programmes under the new area of bioinformatics. Spectral analyses of DNA sequences (R. Ramaswamy, Jawaharlal Nehru University, New Delhi) have revealed interesting periodicities which can allow discrimination between coding and noncoding regions in a sequence of DNA. The underlying bases of such periodicities are still not obvious and understanding these features will go a long way in deciphering the complexity of the language of life. In a different level, the process of reproduction in higher organisms (plants and animals) is one of the most interesting and complex events in nature. Apart from having separate individuals carrying specific male and female reproductive functions, the presence of individuals being polymorphic for reproductive modes (e.g. asexual plant's ability to produce male gametes), has always raised population geneticists' interest (Amitabh Joshi, JNCASR, Bangalore) since this involves considerably high evolutionary cost. The existing models have not been able to give unequivocal reasons for the evolutionary cost of sex in such asexual individuals, and there is a need to look at the arguments and experimental results afresh to find out the consequences of maintenance of such activities in evolutionary scale.

One area in biology which has been using theory successfully for a long time is mathematical ecology. Interestingly, in turn, it has also contributed to the advancements in conceptual understandings in physics and nonlinear dynamics. The mathematical models describing growth of populations have been used in many areas of science such as, demography, economics, biological resource management, and epidemiology and health. The models for discrete population growth, used for annual plants or insects, show very complex dynamics such as period-doublings and chaos. Though erratic oscillations in the size of a population are observed in laboratory-grown organisms, natural population data do not seem to show such complex dynamics when studied for a large number of insects. A simple theoretical study (Somdatta Sinha, CCMB, Hyderabad) showed that common ecological processes like migration can induce stable behaviour in these model populations indicating the possible reason for the observation with natural insect data. Such processes can also show up the finer differences among the models used in ecology and their complex response to ecological processes which can have serious implications in harvesting policies.

Tasks ahead

The meeting had a large canvas. Theoreticians tried hard to underplay the equations and everybody took keen interest in learning from others. At the end of the meeting it was clear that there is much interest and sincere enthusiasm among theoreticians to study biological problems, but they are bewildered by the large amount of experimental details from which they have to strain out the necessary information for modelling. The biologists are interested, but with some apprehension, since they are still not sure where all this leads to. There is a wide gap in understanding each other's motivation, and the type of questions that seem relevant to one do not seem to be so to the other group. This difference can be narrowed down by simply trying to evolve a common language and making a serious effort to have discussions for working on common problems. The consequent cross fertilization of ideas from different branches of science can only enhance the knowledge base and will be useful to all. Because *finally* modelling has to be relevant *biologically*, along with furthering the understanding of complex system dynamics, it is also important for workers with theoretical expertise to put efforts in the direction of possible biological applications in societal and economic fields. Right now there is less than critical mass of workers in this field spread in isolated pockets throughout the vast country. As part of short term measures one can hope to reduce the gap through seminars and workshops involving students/teachers/workers from all fields and strengthen this area of study. For long-term measures, effective communication between the two groups can be enhanced by removing/lowering the artificial barrier created by separating the physical and biological sciences courses with more interdisciplinary knowledge which may increase the ease of interaction among future workers in this area. The major organizational bodies with the Government of India, like the Council for Scientific & Industrial Research, Department of Biotechnology, Department of Science & Technology and the Academies of Sciences have been very supportive in this direction with financial help for organizing meetings. After all, compared to many other scientific fields, theoretical biology can give large rewards with much less financial input. India has shown aptitude for theoretical research in all fields and there is every reason to think that theoretical biology will flourish in this country given the proper environment.

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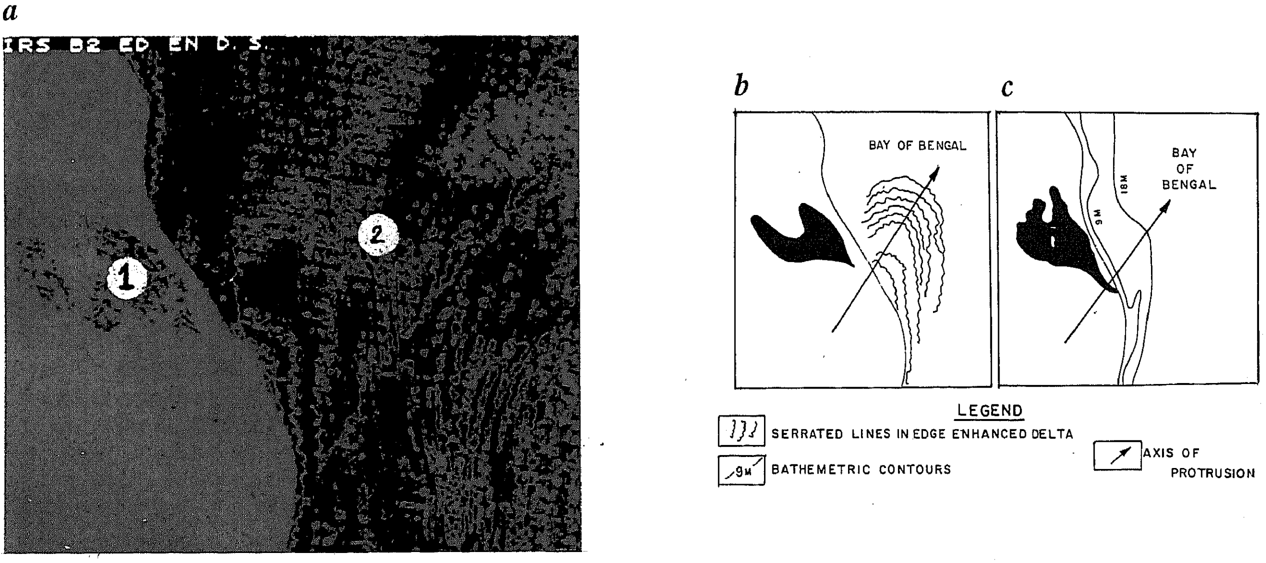
Detection of submarine delta along Madras coast, India, using IRS imagery

Remote sensing has proved its credibility beyond doubt in natural resources survey and environmental monitoring owing to its synoptivity, multispectral photographic capability and repetitive coverage. While its synoptivity gives unbiased information on the regional panorama of the earth surface, its multispectral possibility aids precise interpretation of various objects, as different terrestrial objects exhibit various spectral responses in different bands of the electromagnetic spectrum. Amongst various terrestrial objects, water has a characteristic spectral response of its own, i.e. it permits the blue/red part of EMR (electromagnetic radiation, 0.4–0.8 μm) to penetrate inside. On the contrary, it totally absorbs the infrared part of EMR (0.8–1.5 μm). As such blue/red part of EMR penetrates the water bodies, the impurities and suspended sediments are seen very clearly in various shades of gray as and when the water bodies are shallow. Many workers, therefore, have used blue/red band of the satellite data in such water-quality studies[1-3]. Hardy[4] has observed that the infrared light in the spectral regions of 0.6–1.4 μm has the potential to map the turbidity in the ocean water, whereas Kritakos *et al.*[5] have used the rationed data of visible and near infrared bands (0.4–1.1 μm) and obtained better results for water quality monitoring. Keeping such facts in mind, the blue/red band (band 2) of the IRA 1A satellite data was used to configurate the bathymetry along Madras coast in Bay of Bengal (Figure 1 *a*).

In the present study, the IRS 1A band 2 data (0.52–0.59 μm) of March 1991 was used and subjected to edge enhancement studies for the coastal zone of Madras (Figure 1 *a*). Such edge enhancement normally sharpens the edges of two contrasting objects or sharpens the edges of objects of contrasting relief. In such edge enhanced data, few straight to serrated lines resembling contours were observed in the offshore region of Bay of Bengal in the area east of Pulicat lake (Figure 1 *a*, *b*). These serrated concentric lines were correlated with the bathymetric charts supplied by Survey of India (Figure 1 *c*). This indicated that the serrated lines of the core almost coincided with the 5 fathom contour (equivalent to 9 m depth) and the serrated lines of the outer rim were found to coincide with the 10 fathom line (equivalent to 18 m depth). This suggested that the serrated lines observed in the edge enhanced IRS data (Figure 1 *a*) are nothing but the different relief variations of the continental shelf. Configuration of such serrated lines observed in edge enhanced data as well as the value and shape of the bathymetric contours have thus led to the conjecture of an arcuate positive subaqueous landmass with NE–SW orientation (Figure 1 *b*, *c*).

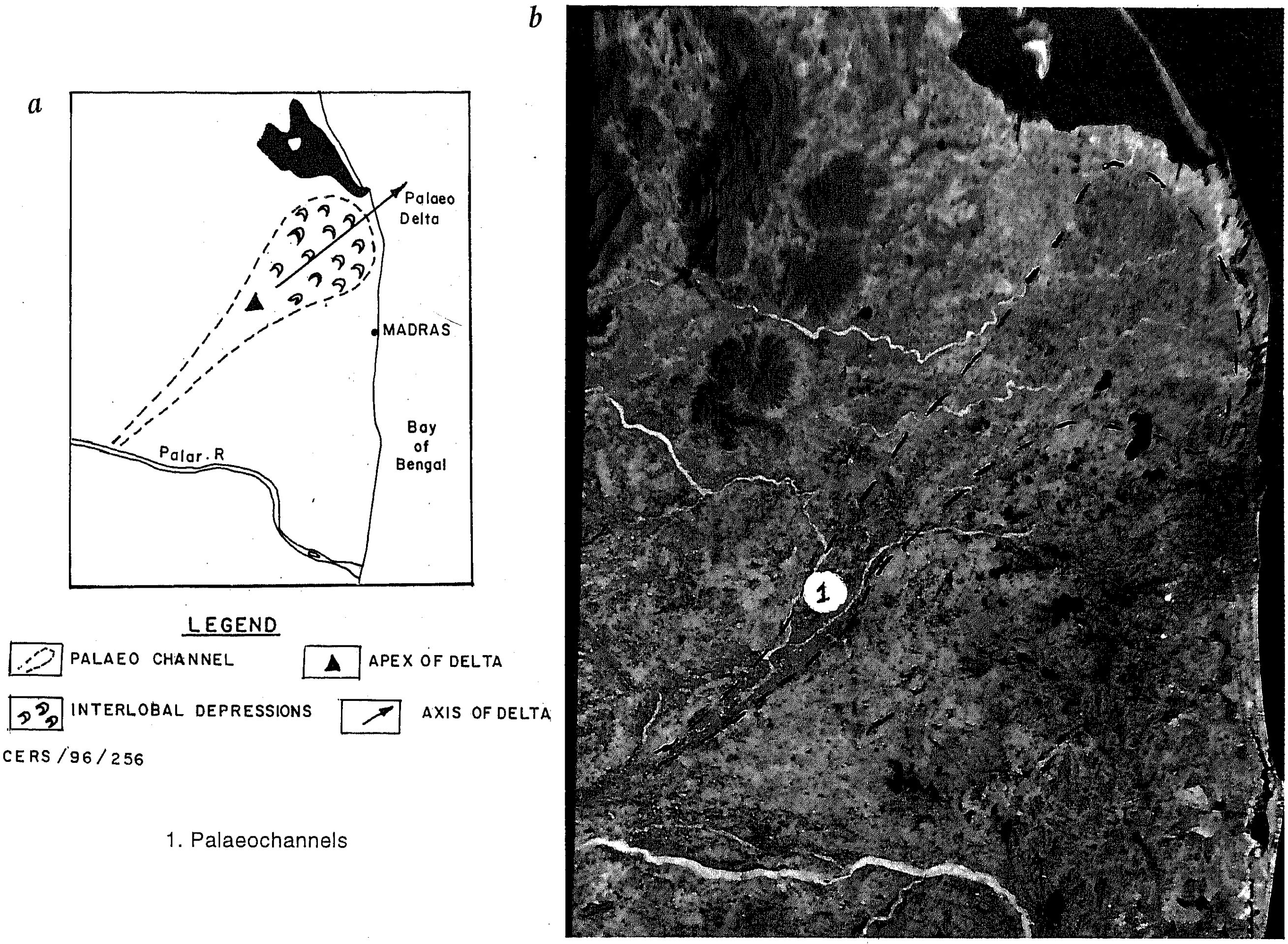
Ramasamy[6] has observed a huge palaeochannel in the area north of Madras (Figure 2 *a*, *b*) and Ramasamy[7] has observed a major lobate delta again north of Madras and demonstrated that both the palaeochannel and the continental delta belong to the Cauvery river which has once flown in the area. He[7] has further aligned the axis of such continental delta and termed the delta as 'proto Cauvery delta' in NE–SW direction. The axis of such proto-Cauvery delta also coincides with the axis of the positive arcuate subaquaous landmass observed in the edge enhanced data (Figure 1 *b*) and the bathymetric data (Figure 1 *c*).

The occurrence of the lobate proto Cauvery delta north of Madras (Figure 2 *a*, *b*) and the positive arcuate subaquaous landmass observed in edge enhanced data and bathymetric charts in the north eastern extension of such



1. Pulicat lake
2. Bay of Bengal

Figure 1.



1. Palaeochannels

Figure 2.

proto Cauvery delta confirms that this delta might have extended up to 20–30 km inside the continental shelf in the area each of Pulicat lake.

Thus the present study reveals the possibility of utilizing blue/red band of the IRS data in ocean bathymetric mapping. In addition, the present study clearly demonstrates the existence of a huge submarine delta east of Pulicat lake, confirming the flow of the mighty Cauvery river in this region in the recent past.

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A new overlay in virological work for animal viruses

Virus assay by plaque method is a basic need in virus laboratories. The virus assay system by plaque method needs pure chemicals. The overlay ingredients/chemicals should not be toxic either to the host system or inhibitory to the virus. Various overlay materials such as agar, agarose, carboxymethyl cellulose, methocil and paraffin oil are

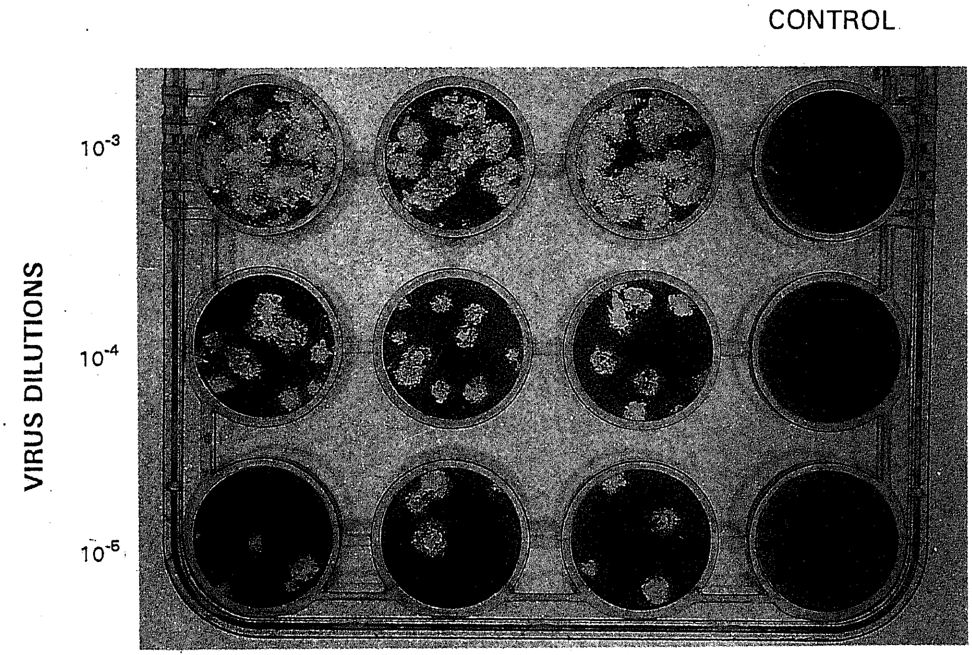

Figure 1. Japanese encephalitis (JE) virus plaques in PS cells under sago overlay showing plaques in different virus dilutions. Control wells without virus.

used extensively. Nene *et al.*'s article provided the inspiration for the present study[1]. Sago was purchased from local market and was used as an overlay on the cells. The sago was powdered in the grinder and stored in a cool and dry place. 7 g of the sago powder was suspended in distilled water and the suspension was mixed on magnetic stirrer for 15 min. This suspension was sterilized by autoclaving 10 lbs/10 min and stored at +4°C. Vero cell monolayers were grown in 24 well plates (Linbro), infected with different viruses like Chandipura, Chikungunya, Japanese encephalitis, polio, etc. and adsorbed for 30 min at 37°C. The plates were washed with medium and one ml of the overlay was added. The overlay composition was as follows:

(i) Sterile sago 7% in distilled water, 50%, (ii) Sterile 2X MEM, 50%; (iii) FCS, 2%.

The plates were incubated in 5% CO_2 atmosphere for appropriate time and the overlay was removed by pouring it in a discarding pan. The wells were rinsed with normal saline and fixed in formal saline for 30 min; after fixation the wells were washed with tap water and stained with 0.1% crystal violet for 30 min and the plates were washed with tap water.

The plaques of these viruses were clear (Figure 1) and there was no inhibitory effect of sago on plaque formation. The only drawback in this overlay was that, it is not as transparent as agar or agarose, however, the cost effectiveness and routine Plaque Reduction Neutralization Test (PRNT) work in virology can be done at low cost. Another advantage is that, this overlay does not require heat to melt, so that many thermolabile viruses can be easily handled without any loss. Our study corroborates the views expressed by Nene *et al.* regarding the cost effectiveness and other beneficial qualities of sago (tapioca).

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Medical research on the sick list

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Evaluation of performance in science is a useful, and at times unflattering, exercise. Bibliometry is an evaluation procedure whose application is new to medical research in India. No wonder Arunachalam's report[1] based on Indian medical papers cited in *SCI* and his conclusion that a substantial mis-match exists between the needs of health in India and the research actually done had evoked considerable interest earlier. As the *SCI* covered just one medical journal from India, Arunachalam has repeated the study by using *Medline* as the source of data because a high proportion of medical papers are published in local or national journals[2]. His painstaking analysis not only confirms the mismatch noted earlier but also uncovers a disturbing picture of medical research in India. Admittedly bibliometry is handicapped, for example, by problems of classification between journals and diseases, correctness of reported data on mortality and morbidity and the bias of editors and indexing agencies. These difficulties do not, however, detract from the main significance of Arunachalam's study. He found that in seven years from 1987 to 1994, Indian authors published 19,916 articles in 1440 journals; of which 14,822 were published in journals whose impact was less than 1.0. Only 58 papers appeared in journals with an impact factor higher than 8.0 and some of these belonged to new biology and not mainstream medical research. Among the published papers, general and internal medicine claimed top position (2602), followed by paediatrics (1420) and pharmacology (1367) with seven other fields claming publications in three-digit figures. However, the top ten fields in Indian medical research did not include tropical medicine or respiratory diseases which rank high according to the mortality and morbidity statistics in India. Again, with more than 9 million blind including two million blind children, hardly any research was done in ophthalmology on the basis of publications. Oncology, cardiovascular and neurological diseases did not figure among the leading causes of mortality and morbidity according to the official data supplied to the WHO; yet 2068 papers were published in these disciplines. Even if some papers in tropical and respiratory diseases had been listed under medicine and paediatrics, and the Indian statistics on mortality and morbidity are not fully reliable, Arunachalam's findings do reveal a lopsided order of priorities in Indian medical research. A less serious but equally disturbing, finding is that no more than a small fraction of the 250 Indian medical journals received by the National Library of Medicine are covered by *Medline*. The analysis also brings out other interesting information such as universities and colleges leading in medical publications; CSIR institutions publishing more than the ICMR group and the DAE institutions excelling those under the Ministry of Health (excluding AIIMS and PGI) in publications.

Though Arunachalam seeks the explanation for the dominance of cancer and cardiovascular research in the affluence of patients, better facilities of tertiary hospitals, etc., the analysis does not get to the heart of the problem. The poor correlation between major health problems and the preferences of investigators has reasons which go deeper into the history and evolution of medicine in India. The originality and spirit of enquiry which characterize *Charaka Samhita* vanished by the early centuries of the Christian era and an age of stagnation began in Indian medical endeavour after the appearance of *Ashtanga Hridaya* in the 7th century. Long starved of new knowledge, India welcomed western medicine in the nineteenth century and quickly learnt to use its tools and methods without bothering to learn how to make the tools and methods. This failure, like a birth defect, became a handicap and ensured that successive waves of tools and methods from the West, and not societal needs, determined the medical agenda in India. In many ways, this is reminiscent of Indian scientific research in general: it remained aloof from the people and failed to power our socio-economic development which continues to progress by infusions of foreign knowhow. Once we begin to devise our tools and methods and apply them for solving the health problems around us, Indian medical research will gain speed and purpose and hopefully, bibliometric acclaim.

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How relevant is medical research done in India? – A study based on *Medline*

Subbiah Arunachalam

Does India perform medical research in areas where it is most needed? According to Government of India sources, India suffers mainly from diarrhoeal diseases, infancy diseases, respiratory diseases, tuberculosis and malaria. An analysis of journal use as seen from seven years of Medline reveals that Indian researchers are active in general and internal medicine, paediatrics, pharmacology, immunology, pathology, oncology, surgery, cardiovascular research, gastroenterology and neurosciences. Apart from analysing the reasons for the mismatch, this study provides inventories of the amount and nature of available expertise and its institutional and geographic distribution.

THIS piece of research is based on the premise that quantitative information on the output of the health research community is a valuable first step in the complex process of improving the contribution health research makes to the solution of a nation's health problems[1], and the belief that scientometric tools, developed and used in the West, can be adapted to study many aspects of scientific activity in India[2]. Apart from trying to quantify medical research in India and evaluating its relevance to the country's needs, I draw attention to the need for exercising caution in using international databases in evaluating indigenous research performance.

Any human endeavour, especially one that involves public funding, should be subjected to assessment and performance review. Research is no exception. For a very long time, scientific research in India was thought to be an inherently good thing deserving public support, and not much attention was paid to assessment and performance review. Those days of idealism are over. Now both the government and the taxpayers, not only in India but around the world, are more pragmatic and they would like to see their investment in research bring in adequate returns.

Indeed the US Congress passed in 1993 the Government Performance and Results Act, and the Clinton Administration initiated in the same year the National Performance Review, which focused on the performance of all government programmes including the assessment of the government's investments in research. In assessing research performance, one is not merely interested in the economic returns and social impact of research but also in the quality of research and its impact on advancement of knowledge.

In the West there has been a tradition of evaluation of research, witness the large number of articles in this area published in journals like *Scientometrics*, *Science and Public Policy*, *The Scientist*, *Science Watch* and *Journal of the American Society of Information Science*. The US National Science Foundation started bringing out its biennial Science Indicators reports (now Science and Engineering Indicators) as early as 1972. A few years ago, the Ciba Foundation organized a symposium on evaluation of research in which the world's leading experts such as Alvin Weinberg, Eugene Garfield, the late Michael Moravcsik and Tibor Braun took part[3]. One aspect, viz relevance of research, I thought, was not given as much attention in that symposium as it deserved.

The question of relevance is especially important in a developing country, where scarce resources have to be used judiciously. The relatively low research expenditure underlines the importance of making appropriate strategic research choices. Also, as pointed out by Hicks *et al.*[1], 'it is important that health research priority setting be developed in tandem with existing research capacity. This underlines the importance of assessing the amount and nature of available expertise and positioning research output, both in the national health needs, and in the international science research contexts[1].'

In early 1995, I looked at the relevance of medical research in India, by looking at the disease pattern as revealed by mortality and morbidity statistics on the one hand and, on the other, the journals used by Indian medical researchers to publish their work as seen from five years of *Science Citation Index* (1981–1985), and came to the tentative conclusion that there was a substantial mismatch between the needs and the areas where work was being done[4]. My findings received considerable attention, perhaps because, while bibliometric analysis has been used for many other purposes, it has rarely been used to evaluate the relevance of a nation's research programme. Besides, few attempts have been made so far to inventory and assess the quantity and

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Table 1. Journals used often by Indian researchers to publish their papers in medical research [Source: *Medline* Nov. 1987– Dec. 1994]

Journal	Country	Subject	Impact factor	No. of papers
Indian Pediatr.	IND	Pediat.	0.000	801
Indian J. Exp. Biol.	IND	Biology	0.000	777
J. Assoc. Physicians India	IND	Med. Gen.	0.000	705
Indian J. Med. Res.	IND	Med. Gen.	0.000	569
J. Indian Med. Assoc.	IND	Med. Gen.	0.000	396
Indian J. Physiol. Pharmacol.	IND	Physiol. //Pharmacol.	0.000	377
Bull. Environ. Contam. Toxicol.	USA	Env. Sci.	0.766	321
Biochem. Int.	AUS	Bioch. Mol.	0.690	314
Indian J. Biochem. Biophys.	IND	Bioch. Mol. //Biophys.	0.000	298
Indian J. Pediatr.	IND	Pediat.	0.000	287
Indian J. Pathol. Microbiol.	IND	Pathology	0.000	279
J. Postgrad. Med.	IND	Med, Gen.	0.000	254
Indian J. Lepr.	IND	Dermatol. //Immunol.	0.000	237
Indian J. Gastroenterol.	IND	Gastro.	0.000	235
Indian Heart. J.	IND	Cardiovasc.	0.000	234
Int. J. Cardiol.	NLD	Cardiovasc.	0.545	186
Indian J. Cancer	IND	Oncology	0.000	167
Mutat. Res.	NLD	Genetics	1.727	159
Indian J. Ophthalmol.	IND	Ophthal.	0.000	152
Biochem. Biophys. Res. Commun.	USA	Bioch. Mol. //Biophys.	3.803	145
Biochem. Biophys. Acta	NLD	Bioch. Mol. //Biophys.	2.460	137
Indian J. Chest Dis. Allied Sci.	IND	Resp. Sys.	0.000	137
J. Commun. Dis.	IND		0.000	135
Indian J. Malariol.	IND		0.000	125
Indian J. Med. Sci.	IND	Med. Gen.	0.000	119
Natl. Med. J. India	IND	Med. Gen.	0.000	108
Mol. Cell Biochem.	NLD	Bioch. Mol.	1.149	103
Indian J. Dermatol.	USA	Dermatol.	0.534	101
Cancer Lett.	NLD	Oncology	1.075	97
Plant Foods Hum. Nutr.	NLD	Nutri. Diet.	0.000	95
Trans. R. Soc. Trop. Med. Hyg.	UKD	Pub. Health	1.175	94
J. Ethnopharmacol.	CHE	Botany //Pharmacol.	0.412	92
J. Laryngol. Otol.	UKD	Otorhino.	0.317	88
Int. J. Lepr. Other Mycobact. Dis.	USA	Pathology	0.000	85
Contraception	USA	Obst. Gyne.	1.038	83
Indian J. Publ. Health	IND	Pub. Health	0.000	83
J. Surg. Oncol.	USA	Oncology //Surgery	0.492	82
Ecotoxicol. Environ. Safety	USA	Env. Sci. //Toxicol.	1.155	78
FEBS Lett.	NLD	Bioch. Mol. //Biophys.	3.479	77
Br. J. Urol.	UKD	Urol. Neph.	0.695	76

quality of existing research capacity, especially in the health sector, in developing countries. I knew all along that it would not be advisable to draw conclusions entirely based on my analysis of the *SCI* data of five years, as *SCI* had covered during the period I took for analysis just one Indian medical journal, viz *Indian Journal of Medical Research*. In medical research, unlike in physics or chemistry, a high proportion of papers was likely to be published in local or national journals.

I decided to repeat the study, this time using the CD-ROM version of the standard edition of *Medline* as my source instead of *SCI*.

Methodology

I collected data from *Medline* 1987 November to 1994 December. I chose a fairly long period to avoid possible misleading conclusions that might arise from deviations that could result from short-term fluctuations in emphasis on research in different medical specialities. As *Medline* does not always include complete addresses of authors, making a search by merely giving 'India' in the address field, one would miss many Indian papers. I included the names of all Indian cities and towns where there are higher educational and medical research institutions. For all entries originating in India, I downloaded the necessary bibliographic data and converted them into a database. The analysis of the data for identifying prolific institutions and cities/towns, journals used for publishing papers from Indian institutions, etc. was carried out using Foxpro.

Problems pertaining to data analysis

There were a few issues to be resolved. The first related to the level of aggregation at which India's contribution to medical literature is classified. Should it be at the individual article level? That would be ideal, but next to impossible. I decided to look at India's contribution at the journal and institutional levels. Following the example of CHI Research, Inc.[5] and ISSRU of the Hungarian Academy of Sciences[6], I allotted whole journals to subfields and subfields to major fields, and classified each paper into the field/subfield of the journal in which it was published. The second concerned the classification of diseases and journals. Diseases are usually classified as pertaining to different systems such as respiratory system, circulatory system, and nervous system, whereas journals are classified under fields and subfields such as allergy, andrology, gastroenterology, and surgery. By and large, I used the classification of journals followed by *SCI* and given in the *SCI Guide*[7]. For non-*SCI* journals, I used the classification used by Ulrich, the well-known reference source on serials literature. The world scientometric community is fully aware of the problems in all kinds of classification. These were discussed at a one-day workshop[8] immediately following the Fifth Biennial International Conference of the International Society of Scientometrics and Informetrics at River Forest, IL, USA, in June 1995. The third problem related to the correctness of the mortality and morbidity data. These were collected from a report of the World Health Organization's South East Asia Regional Office[9], New Delhi, and the report clearly states that the data were provided by Indian agencies and they were reliable to only a certain extent.

Results and discussion

Journals used

In the seven years (as seen from *Medline* Nov. 1987–Dec. 1994), Indian researchers had published 19,952 items in 1440 journals. Of these, 19,916 were journal articles (as classified by *Medline*), nine were letters and eight clinical trials.

The 40 journals in which Indian researchers had published at least 75 papers in the seven years are listed in Table 1, along with the number of papers published in each one of them, the journal country and the subject category to which the journal belongs. Some journals belong to more than one medical subfield, e.g. *Indian Journal of Leprosy* is classified under three subfields, viz. dermatology, immunology and pathology. At the time of writing, a few journals were left unclassified, as they are neither indexed in *SCI* nor are they listed in the print version of Ulrich. Table 1 also gives the impact factors of the journals taken from *Journal Citation Reports* 1991. An impact factor of 0.0 means that the journal is not indexed in *SCI*.

Of these 40 journals, 21 are published in India. The top six in this list as well as 13 out of the top 15 are Indian journals. There were nine other Indian journals (not included in Table 1) from which *Medline* had indexed at least one paper during the period studied. These are: *Trop. Gastroenterology* (69 papers), *Hindustan Antibiotics Bulletin* (33), *Journal of the Indian Society of Pedod. Prev. Dent.* (28), *Indian Journal of Dental Research* (27), *Journal of the Pierre Fauchard Academy* (14), *Fed. Oper. Dent.* (12), *Journal of the Indian Dental Association* (11), *Indian Journal of Dermatology* (9), and *Acta Anthropogenetica* (6). Thus *Medline* had covered thirty Indian journals during the period under study. Not all 30 were, however, covered in each one of the seven years. For instance, in 1992, it covered only 22 of them. Three of the 30 Indian journals indexed in *Medline* (viz. *Indian Journal of Biochemistry and Biophysics, Indian Journal of Experimental Biology* and *Acta Anthropogenetica*) are not mainstream medical journals.

Most Indian papers indexed in *Medline* had appeared in low-impact journals. Nearly three-fourths (14,822 out of 19,952) were published in journals whose impact factor (*JCR* 1991) was less than 1.0 or in journals which were not indexed in *SCI*. These include 9,525 papers in 530 non-*SCI* journals (impact factor taken to be 0.0) and 5,297 papers in 419 journals with impact factor less than 1.0. Only 58 papers were published in journals whose impact factor was higher than 8.0. Besides, some of these papers in high impact journals, such as the 12 papers in the *Proceedings of the National Academy of Sciences USA*, may not be mainstream medical research papers and are most likely to be in related areas such as new biology/biomedical research.

Analysis by subfield

Medline covers not only the literature of medicine, but also related fields such as biochemistry, biophysics and even chemistry and materials science (biomaterials). In Table 2, I list only papers that are classified under 45 subfields of medicine. In these subfields, authors from Indian institutions have used 1013 journals to publish 13,855 articles. As some journals are included in more than one subfield, totalling by subfield leads to 1368 journals and 18,244 articles as seen from Table 2. There is a large difference between the two sets of numbers. Take, for example, pharmacology. If we include Indian articles in all journals under the subfield, there are 1367 papers published in 94 journals. But, if we avoid duplicate counting, the number of journals drops to 53 and articles to 398. There is a reason for this large drop: whenever a journal is classified under pharmacology and one or more other categories, pharmacology is the one which gets left out as most of the time the subfields under which a journal falls are arranged alphabetically. In many cases, the additional category is not among the 45 subfields listed in Table 2 (see note 1).

Table 2. Indian research papers covered by *Medline* Nov. 1987–Dec. 1994 classified by subfields

Subject	No. of journals	No of papers	No. of journals	No. of papers
		(No duplicates)		
Med. Gen.	57	2394	52	2374
Pediat.	43	1420	33	1367
Pharmacol.	94	1367	53	398
Immunol.	74	928	61	534
Pathology	48	916	21	465
Oncology	56	821	39	692
Surgery	68	750	35	335
Cardiovasc.	41	663	41	663
Gastro.	26	606	25	537
Neurosci.	101	584	88	513
Pub. Health	46	569	27	405
Toxicol.	42	568	16	207
Microbiol.	52	553	32	365
Dermatol.	25	551	24	516
Physiol.	30	533	16	445
Trop. Med.	13	432	6	152
Obst. Gyne.	37	417	31	386
Radiol.	52	403	34	277
Ophthalmol.	28	362	25	342
Vet. Med.	30	302	22	178
Endocr.	41	300	34	233
Parasitol.	20	292	15	251
Urol. Neph.	25	283	21	258
Resp. Sys.	17	280	12	258
Nutri. Diet.	20	278	15	229
Med. Res.	38	208	25	146
Psychiat.	30	184	22	152
Dentistry	29	169	26	162
Anatomy	20	149	20	149
Otorhino.	16	135	15	131
Orthoped.	20	122	17	101
Virology	10	97	9	91
Allergy	8	92	8	92
Med. Mis.	16	88	14	76
Andrology	4	82	4	82
Hematol.	20	77	17	60
Anesthes.	11	67	9	61
Psychol.	19	46	11	25
Med. Leg.	7	39	7	39
Geriatrics	8	35	7	34
Med. Lab.	7	27	7	27
Rheumatol.	7	27	7	27
Sub. Abuse	9	24	7	16
Epidemiol.	1	2	1	2
Nursing	2	2	2	2
Total	1368	18244	1013	13855

*Whenever a journal is included in more than one subfield, to avoid duplicate counting, it is taken into account under only one category (which is given as the first subfield in Table 1); often, this happens to be the category which has alphabetical precedence.

Indian researchers have used 101 journals to publish 584 papers in neurosciences, and 94 journals to publish 1367 papers in pharmacology. In contrast, at the other end of the spectrum, they have used just one epidemiology journal to publish two papers, and two nursing journals to publish two papers. In terms of number of papers published, general medicine tops the list with 2,394 papers. Research medicine (208 papers) and general medicine (2,394 papers) together account for 2,602 papers. One can take this number as India's contribution in the area of general and internal medicine. Paediatrics comes next with 1,420 papers, followed by pharmacology (1,367), immunology (928), pathology (916), oncology (821), surgery (750), cardiovascular research (663), gastroenterology (606) and neurosciences (584).

The top ten fields in Indian medical research, in terms of number of papers published, do not include tropical medicine and respiratory system diseases, two areas which are very important in India, as seen from mortality and morbidity statistics (Table 3). Cancer/oncology and cardiovascular diseases do not figure in Table 3, and yet relatively considerable amount of research is being carried out in India in these areas. Again, with more than nine million blind, including two million children, India tops the world in the incidence of blindness. India also suffers from a very high incidence of glaucoma and cataract. But, there is hardly any research in ophthalmology.

Analysis by journal country

In Table 4, I have classified Indian papers indexed in *Medline* by journal country. The number of Indian journals covered by *Medline* is only a small fraction of the more than 250 Indian journals received at the National Library of Medicine, National Institutes of Health, Bethesda, MD, USA, the publishers of *Medline*. Thus, while *Medline*'s coverage of Indian journals is better than the coverage in *SCI*, which covers only one medical journal from India, viz. *Indian Journal of Medical Research*, it also leaves out a very large number of Indian journals. The fact is, simply, many Indian journals do not meet the criteria for inclusion in *Medline*. Editors and publishers of Indian journals should examine why Indian journals do not measure up to the expectations of

Table 3. Leading causes of mortality and morbidity in India by rank order

1983–1985	1988–1990	1991–1993
	Leading causes of mortality	
Senility	Infectious and parasitic diseases	Diarrhoeal diseases
Respiratory diseases	Circulatory system diseases	Respiratory diseases
Infancy diseases	Respiratory diseases	Infancy diseases
Circulatory diseases	Injury/poisoning	Pneumonia
Fevers	Diarrhoeal diseases	Infectious and parasitic diseases
	Leading causes of morbidity	
Data not available	Diarrhoeal diseases	Respiratory diseases
	Influenza	Diarrhoeal diseases
	Malaria	Malaria
	Tuberculosis	Whooping cough/Measles
	Whooping cough	Neonatal tetanus

Source: *Health Situation in the South-East Asia Region 1991–1993*, World Health Organization Regional Office for South-East Asia, New Delhi, 1995, pp. 40–41.

international database producers. Samiran Nundy and colleagues at the All India Institute of Medical Sciences have carried out a study on the quality of Indian medical journals and they found that most of them are poor[10,11]. On a request from the Indian Council of Medical Research, Nundy and colleagues examined 113 serious English-language journals published in India covering a wide range of subjects such as anaesthesiology, genetics, parasitology, social and preventive medicine and urology[11]. Most of these journals were sponsored by professional societies and academic bodies and all but a handful were not coming out on time and only a few were indexed in international secondary services (three in *Current Contents*, 22 in cumulated *Index Medicus* and 26 in *Excerpta Medica*). Says Nundy[10]: 'most journals not included in the international indexing services did not deserve to be included.'

Nundy and co-workers have also found from an analysis of the publication output of more than 125 Indian medical institutions, as seen from eight years of *SCI*, that most of these institutions are not active in research[12]. This view is in general agreement with the opinion of M. S. Valiathan, who points out that India has hardly contributed anything to modern surgery[13]. Noting that starting from the late nineteenth century a number of Indian physicians and surgeons had gone to Britain, and lately to the US as well, for training and that many of them had won respect and acceptance all over the world for their competence and universality of outlook, Valiathan wonders why, despite all the achievements, 'India's name did not figure in the honour roll of nations which contributed to the advancement of surgical knowledge despite her wholehearted adoption of European medicine and surgery.' In his view, 'India enjoyed a free ride in surgery from the nineteenth century, borrowing Western theory and practice and contributing nothing.' Valiathan is emphatic in asserting that 'in surgery India lives on borrowed intellectual capital' and that 'no concept, no discovery, no technology or procedure originated in India which shaped or directed the course of global surgery[13].'

Overall, Indian researchers use Indian journals the most, followed by US, UK, Dutch, German and Swiss journals (Table 4). A very large percentage of Indian papers in paediatrics (1,088 out of 1,420), general medicine (2,151 out of 2,394) and physiology (377 out of 533) had appeared in Indian journals. In tropical medicine, an area of considerable importance to India, Indian research publications had appeared mainly in British journals: 69 in Indian journals and 238 in UK journals. In neurosciences, India had published all her papers in foreign journals, mostly in US and UK journals.

Use of British vs American journals

There were six papers in *British Medical Journal* and 22 in *Lancet* in my *Medline* sample, but none at all in the *Journal of American Medical Association* and only one in the *New England Journal of Medicine*. There were two papers in *Nature* and one in *Science*. Two points are evident. One, not all papers published in *Nature* and *Science* are indexed in *Medline*. This is understandable as those not indexed may not pertain to medicine. What is surprising is that not all papers from India in *Lancet* are also being indexed in *Medline*! For example, *SCI* 1992 (CD-ROM version) has indexed 30 papers published in *Lancet*, from India[14], but *Medline* has indexed only 22 in seven years. Two, it appears to be far more difficult for Indian researchers to get their papers published in leading American journals than in British journals. Data from four years of *SCI* (1989-1992) show this fact not only with respect to *Lancet* and *NEJM* but also with respect to *Nature* and *Science*[14]. There could be many reasons, such as page charges levied by American journals, editors' attitudes and India's historical links with the UK. Editors' attitudes do matter. The editor of the *Lancet* Richard Horton, the former editor of *BMJ*, Stephen Lock and the current editor Jane Smith are known to be sympathetic to Third World researchers. The *BMJ* editors have come to India more than once to exchange views with Indian editors and to conduct training programmes on medical writing. Richard Horton, as chair of the World Association of Medical Editors, assembled a global network of researchers to assist editors of Third World journals in establishing peer review processes. Horton believes that often cultural differences are misinterpreted as bad science[15]. In contrast, the editor of *New Engl. J. Med.*, Jerome P Kassirer is on record as having said that 'what developing countries should receive is guidance on nutrition and immunizations before getting advice on medical editing'. He says

Table 4. India's contribution to the journal literature of medicine arranged by country of publication of the journals [Source: *Medline* Nov. 1987–Dec. 1994]

Journal country	No. of journals	No. of papers
India	30	6684
USA	509	4428
United Kingdom	314	3158
Netherlands	91	1688
Germany	121	891
Switzerland	81	638
Australia	20	603
Denmark	30	312
Japan	45	251
Czechoslovakia	13	235
Italy	45	232
Sweden	16	170
Canada	14	86
Hungary	9	85
France	25	74
Belgium	8	66
Spain	4	51
Austria	4	49
Thailand	2	46
Poland	10	37
Singapore	3	28
Bangladesh	1	25
Norway	5	19
Greece	2	15
Brazil	5	11
Ireland	5	9
Israel	3	7
Chile	1	6
China	2	6
Korea	2	5
Mexico	2	5
Pakistan	1	5
South Africa	3	4
Hong Kong	1	3
Sri Lanka	1	3
New Zealand	1	3
Yugoslavia	2	3
Costa Rica	1	2
Finland	2	2
Venezuela	1	2
Bulgaria	1	1
Egypt	1	1
Kenya	1	1
Malaysia	1	1
Romania	1	1
Total	1440	19,952

Table 5. Contribution made by different types of institutions as seen from *Medline* (Nov. 1987–Dec. 1994)

Academic	13,111		
Research	3805		
Ministries	1055		
Others	1643		
State	183		
Private	151		
International	4		
	19,952		
Academic			
College		*University*	
Medical	4812	General	4354
General	194	Medical	194
Engineering	129	Agriculture	390
Agriculture	17	Engineering	14
	5152		4952
Research Institutions			
Council of Scientific and Industrial Research			1578
Indian Council of Medical Research			1007
Dept. of Atomic Energy			874
Defence Research and Development Organization			215
Indian Council of Agricultural Research			131
			3805
Ministries			
Health and Family Welfare	642	Home	10
Science and Technology	317	HRD	8
Planning	35	Steel and Mines	2
Industry	27	Env. and Forest	1
Agri. and Rural Develop.	11	Finance	1
		Labour	1
			1055
Others			
Hospitals and Clinics	1650		
General	144		
	1794		
State			
Health and Family Welfare	137		
Home	30		
Industry	13		
Steel and Mines	2		
Public Health	1		
	183		
International (ICRISAT)	4		

that 'very poor countries have much more to worry about than doing high quality research', and that 'there is no science there'[15]. Similar is the attitude of Floyd E. Bloom of *Science*. For him, poor language skills also mean poor science! 'If you see people making multiple mistakes in spelling, syntax and semantics, you have to wonder whether when they did their science they weren't also making similar errors of inattention'[15].

Table 6. Indian institutions often publishing papers [Source: *Medline* Nov. 1987–Dec. 1994]

Institution	No. of papers
All-India Institute of Medical Sciences, New Delhi	1630
Post-Graduate Institute of Medical Education and Research, Chandigarh	1383
Banaras Hindu University, Varanasi	635
Tata Memorial Centre and Cancer Research Institute, Bombay	512
Christian Medical College and Hospital, Vellore	493
Industrial Toxicology Research Centre, Lucknow	383
Indian Institute of Science, Bangalore	369
Central Drug Research Institute, Lucknow	357
Indian Institute of Chemical Biology, Calcutta	266
King Edward Memorial Hospital, Bombay	255
Maulana Azad Medical College, New Delhi	254
Kasturba Medical College, Manipal	248
University of Madras, Madras	235
K. G. Medical College, Lucknow	234
Panjab University, Chandigarh	231
National Institute of Mental Health and Neurosciences, Bangalore	229
Jawaharlal Nehru University, New Delhi	227
Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow	226
Bhabha Atomic Research Centre, Bombay	221
Medical College, Rohtak	214
Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry	214
G. B. Pant Hospital, New Delhi	213
Calcutta University, Calcutta	200
Seth G. S. Medical College, Bombay	199
Sree Chitra Tirunal Institute for Medical Sciences, Trivandrum	187
University College of Medical Sciences, Delhi	174
University of Delhi, Delhi	173
Centre for Cellular and Molecular Biology, Hyderabad	170
Calcutta Medical College and Hospital, Calcutta	145
Punjab Agricultural University, Ludhiana	145
National Institute of Immunology, New Delhi	142
Lady Hardinge Medical College, New Delhi	141
St. John's Medical College, Bangalore	135
Lokmanya Tilak Municipal Medical College and General Hospital, Bombay	134
Haryana Agricultural University, Hisar	133
Institute for Research in Reproduction, Bombay	126
Jawaharlal Nehru Medical College, Aligarh	125
Bose Institute, Calcutta	125
Safdarjang Hospital, New Delhi	116
Indian Council of Medical Research, New Delhi	115

The academic links are also equally important. For historical reasons, many Indians go to Britain for higher education in medicine and unlike those who go to the USA most of them return to India to set up practice and pursue research. Indian students use many British textbooks, many of them available as ELBS low-cost editions. The British Council libraries located in major Indian cities have excellent collections of medical books, journals and reference sources, and doctors form a substantial segment of the membership.

Analysis by institution

The distribution of papers by institutional type is given in Table 5. Academic institutions (universities and colleges) are the leading publishers of medical research papers. Central government institutions have published more papers than state government institutions. Interestingly, Council of Scientific and Industrial Research laboratories have published more papers than Indian Council of Medical Research (ICMR) laboratories. But then ICMR receives very little funding. The Department of Atomic Energy (DAE) accounts for more papers than the institutions under the Ministry of Health and Family Welfare. This is not only because DAE is a far more high profile outfit but also because it has two institutions, viz. Tata Memorial Centre and Cancer Research Institute, Bombay, which publishes a large number of papers, and Bhabha Atomic Research Centre, Bombay, the flagship of DAE, which also contributes a good deal in the area of radiology and nuclear medicine.

Indian institutions which had contributed more than 115 papers in the period under study are listed in Table 6. Only two institutions, viz. All India Institute of Medical Sciences, New Delhi, and Post Graduate Institute of Medical Education and Research, Chandigarh, had published more than 1000 papers each in the seven years studied, and two more, viz. Banaras Hindu University, Varanasi, and Tata Memorial Centre and Cancer Research Institute, Bombay, had published more than 500 papers. Seven other institutions have published more than 250 papers each.

Analysis by city

The distribution of papers by city is given in Table 7. Only cities which have published more than 100 papers are listed. Delhi (including New Delhi) tops the list with 4021 papers. This is largely due to the concentration of institutions performing research in the capital and their proximity to funding agencies, almost all of which are also located in the same city. Why should there be so much concentration of medical research centres in the nation's capital? Sane voices like that of Nundy advocate decentralization of health care and health research facilities. The major institutions contributing to medical research in Delhi are the All India Institute of Medical Sciences, Maulana Azad Medical College, Jawaharlal Nehru University, G. B. Pant Hospital, University College of Medical Sciences, University of Delhi, and National Institute of Immunology. Note that both Jawaharlal Nehru University and University of Delhi figure in the list largely because of their contribution to biomedical research which are also indexed in *Medline*; in fact, their contribution to medical research proper is rather meagre. The same is the case with the Indian Institute of Science, the seventh leading Indian institution in terms of number of papers indexed in *Medline*. Delhi is followed by Bombay, Chandigarh, Calcutta and Lucknow, all of which had contributed more than 1,250 papers each in the seven years. The other major contributors are Bangalore, Hyderabad, Madras, Varanasi, Vellore and Thiruvananthapuram. Delhi, Maharashtra, Uttar Pradesh and West Bengal are the leading states. Maharashtra and West Bengal owe their positions largely to Bombay and Calcutta respectively, whereas contributions from Uttar Pradesh come from Lucknow, Varanasi and Aligarh.

Table 7. Indian cities' and states' contributions to the world literature of medicine as seen from *Medline* (Nov. 1987–Dec.1994)

City	No. of papers	State	No. of papers
Delhi	4021	Delhi	4021
Bombay	2268	Maharashtra	2823
Chandigarh	1654	Chandigarh	1654
Calcutta	1490	West Bengal	1581
Lucknow	1253	Karnataka	1463
Bangalore	886	Tamil Nadu	1319
Hyderabad	671	Andhra Pradesh	910
Madras	663	Kerala	511
Varanasi	648	Punjab	418
Vellore	494	Haryana	397
Trivandrum	418	Madhya Pradesh	362
Pune	373	Gujarat	360
Pondicherry	324	Pondicherry	324
Manipal	272	Rajasthan	318
Ludhiana	261	Jammu & Kashmir	168
Rohtak	222	Bihar	150
Aligarh	216	Orissa	135
Mysore	169	Himachal Pradesh	110
Ahmedabad	165	Meghalaya	68
Hisar	143	Goa	60
Srinagar	129	Assam	39
Agra	127	Manipur	8
Gwalior	125	Tripura	3
Tirupati	114		
Madurai	109		
Baroda	106		
Jaipur	95		
Amritsar	90		
Nagpur	84		
Patna	77		
Allahabad	72		
Shillong	68		
Jabalpur	67		
Mangalore	65		
Patiala	65		
Kanpur	59		
Shimla	58		
Bambolim	57		
Bhubaneswar	57		
Meerut	57		
Indore	54		
Jammu Tawi	53		
Udaipur	51		
Jodhpur	50		

Comparison of analysis based on *SCI* and *Medline*

While by and large the conclusion drawn from the earlier study based on *SCI* data[4] – that a large part of the medical research carried out in India is not in areas where research is needed the most – is validated by this study, there are a few significant differences. In Table 8, I list different subfields of medicine in which India is active in research. There are three lists in descending order of number of papers from India, the first two based on medical journals (under 45 subfields) indexed in *Science Citation Index* in two different periods, and the third based on *Medline* data discussed in this paper.

1. The *Medline*-based study, unlike the *SCI*-based study, indicates that India performs considerable amount of research in paediatrics, an area where much research is truly warranted by mortality statistics. This is largely because most paediatrics papers from Indian institutions have appeared mainly in two Indian journals, viz. *Indian Pediatrician* and *Indian Journal of Pediatrics,* which are covered by *Medline* but not by *SCI*. Another area of considerable importance to India where research is shown to be done in India by our analysis of *Medline* data is gastroenterology. This area did not figure as an active area in our analysis of *SCI* data[4] (see Table 8).

2. The *SCI* data showed that India was doing relatively well in tropical medicine[4], but in the *Medline* data, tropical medicine is not among the top ten fields, in terms of number of papers published. In the *Medline* ranked list, it has dropped to the 16th place; it holds the sixth rank in *SCI* 1981-1985 list and fifth rank in the *SCI* 1991-1993 list (see Table 8). While general and internal medicine and pharmacology occupy roughly the same ranks, viz. within the top three positions, there is considerable variation in the ranks of some fields, e.g. microbiology, pathology and neurosciences. While some of these differences could be attributed to shifting emphasis over time, one cannot ignore the effect of the

Table 8. The relative position of different subfields in terms of number of papers published in three different periods as seen from *SCI* and *Medline*

SCI 1981–1985		*SCI* 1991–1993		*Medline* 1988–1994	
Subfield	No. of papers	Subfield	No. of papers	Subfield	No. of papers
Medicine, General	1640	Medicine, General	817	Medicine, General	2394
Microbiology	976	Pharmacology	665	Pediatrics	1420
Pharmacology	935	Immunology	419	Pharmacology	1367
Endocrinology	367	Surgery	342	Immunology	928
Radiology	301	Tropical Medicine	341	Pathology	916
Tropical Medicine	290	Oncology	322	Oncology	821
Neurosciences	263	Neurosciences	317	Surgery	750
Hygiene	249	Microbiology	311	Cardiovascular	663
Oncology	240	Toxicology	294	Gastroenterology	606
Surgery	226	Cardiovascular	294	Neurosciences	584
Parasitology	202	Pathology	256	Public Health	569
Physiology	186	Radiology	227	Toxicology	568
Pathology	172	Public Health	212	Microbiology	553
Obstetrics	163			Dermatology	551
Immunology	154			Physiology	533
				Tropical Medicine	432

degree of comprehensiveness with which a database covers Indian research in different fields.

It is important, therefore, to know the limitations of the databases used before we draw conclusions!

The interest in cancer and cardiovascular research has another dimension. Although these are not diseases that affect most people in India, i.e. relative to other diseases such as respiratory, diarrhoeal and infectious and parasitic diseases, there are enough rich patients with these ailments who are willing to pay any amount in selected urban hospitals. Doctors in such hospitals are better endowed to carry out research and publish their findings. Many of them have had overseas training, especially in the UK and the USA, where cancer and cardiovascular diseases are rather important. Also, unlike in less expensive hospitals, doctors in such hospitals are not overworked and they can find time to do research and write up their findings.

Lack of co-ordination

Overall, researchers seem to enjoy a lot of freedom in the matter of choice of research problems. There seems to be no co-ordination by an apex agency on what is being pursued. Analysing data from *Medline* on medical research publications from India's southern state of Kerala, Kartha and Mohandas[16] have come to similar conclusions: 'There is a striking contrast between major health problems and those that attract attention of researchers. While infection, parasitic diseases, perinatal and pregnancy-related problems, skin diseases, respiratory diseases and nutritional disorders are the major health problems in the state, a large number of publications are related to cardiovascular problems and cancer. This may be because there are three institutions completely devoted to these disciplines. Even in these areas it is debatable whether research efforts are matched with the needs of the beneficiaries.' Echoing Valiathan's concern on the lack of original contributions from India, Kartha and Mohandas[16] lament that 'simple diagnostic tests for case detection, new modalities for treatment, strategies for identifying high risk population for a specific disease, or methods for prevention of a disease have not been so far originally reported from Kerala'. In their view, 'emerging health problems related to substance abuse, behavioural, environmental and occupational diseases, and mental health problems have not attracted the attention of investigators. Research efforts to a large measure appear to be along beaten tracks, thus leading to very few facts of strategic value being collected'[16].

Conclusion

What Valiathan[13] has said in the context of surgery may very well apply to all of medicine, and what Kartha and Mohandas[16] have stated out of their experience in Kerala may very well apply to all of India. As Samiran Nundy points out succinctly, Indian health care is not good and we should try and improve it; we want our medical profession to have higher standards; and we should not copy Western countries, but do research into our own problems and spend a little more money on health[17]. Laments Sunil Pandya[18], a Bombay-based neurosurgeon: 'Clinical research in India is woefully deficient and inadequate in spite of the availability of an almost unmatched reservoir of patients and illnesses, i.e. clinical and pathological material.' The most important reason for this, says Pandya, is the absence of a culture of research[18]. It would be useful to investigate the role played by professional bodies such as the Indian Medical Association, the Indian Medical Council, and the government's apex agency for medical research, the Indian Council of Medical Research, in orienting research in the country.

Fifty years after Independence is an opportune time to look back and take stock of things. What has been the role of indigenous scientific research in the creation of new and useful knowledge and in finding solutions to problems? It will be instructive to compare research performance in different sectors, especially with agriculture – which like medicine draws heavily on the life sciences. Agriculture research in India has certainly played a key role in transforming a food-deficient country into a food-surplus country. In contrast, medical research in India, but for a few exceptions such as Sambhu De's work on cholera[19] and the development of synthetic heart valves and bloodbags at the Sree Chitra Tirunal Institute[20] – both of these being examples of outstanding work relevant to the needs of India – has not covered itself with glory. This despite the fact that medicine enjoys a better status and image than agriculture in the Indian society. How can one explain this vast difference?

The lack of leadership and the lack of clarity of the goals may be important reasons. Nundy has drawn attention to another facet of this problem, viz. the nexus formed between self-seeking doctors and 'powerful' politicians, seriously harming academic standards in medical education and research[21]. In contrast, ICAR had the benefit of some excellent political, administrative and scientific leaders who had both the vision and the commitment to achieve the goals and the capacity to work together. They could articulate their ideas well and motivate the rank and file. Besides, the country's goals on the food and agriculture front were well defined and well understood, and the Indian farmer, with his abundant common sense, contributed a great deal to the success on the food front. In India, problem solving is not done in medicine as well as it is done in agriculture, and what is achieved in research – say, for example, in immunology – has very little influence on health care delivery. Matching ICMR's research programmes and what is being published by Indian medical researchers in Indian journals on the one hand with their relevance to the health care delivery objectives of the Department of Health and Family Welfare on the other would be revealing. Things might take a while to change. Delivering the Dr Y. Nayudamma memorial lecture in December 1996, J. S. Bajaj, member of India's Planning Commission looking after health-related issues, said that AIDS, cancer, tuberculosis, hepatitis and malaria in that order would be the thrust areas for research, while admitting that communicable diseases, disorders due to nutritional deficiencies and pregnancy-related illnesses would continue to be major causes of morbidity and mortality in the first decade of the 21st century.

One may also refer to ICMR's attitude to research on medical research in India. Arora *et al.*[22] reported in their paper on the best medical colleges in India that ICMR was unwilling to give them information on grants given to medical colleges (on the grounds that it would serve no purpose). My own limited experience in trying to get data on library and information budgets for a study on their impact on research performance was no better: ICAR sent the data within a few weeks for most of its laboratories and ICMR has not sent it yet.

One other problem deserves attention. The peer review process, the very linchpin of the scientific and scholarly enterprise, does not function all that well in India. But the situation in medicine seems to be worse than in other fields. How else can one explain a Nobel-class researcher like Sambhu Nath De, who made, according to P. Balaram, Editor of *Current Science*, not one but at least three major discoveries in cholera and diarrhoeal research which forever altered the fields, going unsung and being little known in the Indian scientific community in his lifetime?

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Note 1. For example, the Swiss journal *Agents and Actions* is covered under chemistry and pharmacology. The six papers published by Indian authors in this journal are not included in the 13,855. Unincluded, similarly, are the six articles in the German journal *Archives der Pharmazie (Weinheim)* and the 27 papers published in the Belgian journal *Archives Internationales de Pharmacodynamie et de Therapie*, both of which are covered under chemistry, which is not covered in Table 2. Again the 92 papers Indian authors had published in the Swiss journal, *Journal of Ethnopharmacology* do not figure in the 13,855 papers, as this journal is classified under botany, another subject not included in the 45 subfields shown in Table 2. However, these missing articles are captured in lists of journals under appropriate subfields, not minding duplication. For want of space, these lists are not shown here.

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Communication signals and sexual selection in amphibians

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Female mate choice is an important determinant for male reproductive success in anurans. The advertisement call of the males contains information for species recognition. These calls are used by the females to distinguish between heterospecifics and conspecifics and further to discriminate among conspecifics to choose the fittest male for the purpose of mating. In *Polypedates leucomystax*, the female responds by its feeble reciprocal call to the first calling male of the colony, which also is the largest and heaviest male amongst all other calling males. This male calls persistently throughout the night, or till amplexus is reached, without changing its call pattern. It increases the intensity of its call after the response of the female. The increase in the intensity, increase in the length of the individual call and persistent calling throughout the night make the call of this first calling male conspicuous. The call contains more acoustical energy which is indicative of good physical condition and the responding female chooses this male. Thus mating in *P. leucomystax* is nonrandom and influenced by female mate choice.

FEMALES of various groups of vertebrates use communication signals produced by the males to choose the fittest male for the purpose of mating. It was this selection pressure which resulted in sexual signals of males to contain information far in excess than that was needed for species recognition. Male sexual displays allowed females to pick more vigorous males and this female choice thus caused further elaboration of male sexual displays[1]. The idea was further extended by Fisher's theory of Runaway Selection[2]. Thus there exists an important relation between communication signals and sexual selection.

Female mate choice has been demonstrated as an important determinant of male reproductive success in anurans. The advertisement calls[3] produced by the males, contain information encoded spectrally and/or temporally important for species recognition[4]. These calls potentially provide a receptive female with information pertaining to species identity, sex and reproductive fitness of the advertising male[5–8]. These signals are used by the females to distinguish between heterospecifics and conspecifics, and further, to discriminate among conspecifics. Based on these informations, the female decides to accept or not to accept the calling male as a potential mate.

Till recently it was believed that it was for the male frogs to produce advertisement calls and act as senders, while the females act as receivers of the acoustic signals to choose the fittest male. As a result, most works were focused on male vocalization and female phonotaxis experiments. Female vocalization due to its feebleness and the secretive nature of the female, had mostly escaped the attention of researchers.

Female reciprocal call

The significance of female reciprocal call in the breeding biology of amphibians was highlighted by Roy *et al.*[9]. They demonstrated the 'catalytic' role of the female reciprocal call for the enhancement of the reproductive activity of the breeding colony. While studying anuran acoustic communication in northeast India and comparing male advertisement call with female reciprocal call in *Limnonectes limnocharis, Euphlyctis cyanophlyctis and Polypedates leucomystax* (Figures 1, 2 and 3), many interesting observations were made. Out of the 3 species, *P. leucomystax* due to its perching habit and its responsive, active and alert nature, was chosen for in depth study of its communication signals and breeding behaviour.

Reciprocal call and courtship display in *Polypedates leucomystax*

The role of female reciprocal call has been studied in detail in *P. leucomystax* for 3 consecutive years (1994–1996) both in the field and laboratory. These frogs are mostly found perched on creepers which entwine bamboo fencing or on tall grass in the vicinity of water. Their perching habit makes them easier to observe than terrestrial or aquatic species and to follow their elaborate breeding behaviour (Figure 4 *a*, *b*).

Weather condition seems to play an important role on the timing of beginning of the first male advertisement call. On dry hot days with temperature ranging between 26 and 37°C, the first male call is heard around 1800 h. On

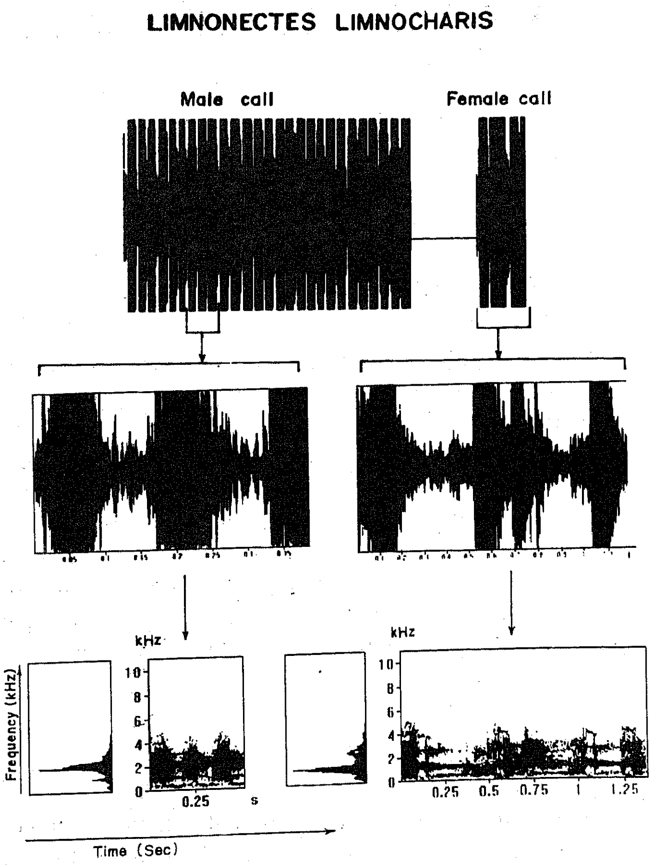

Figure 1. *Top*, Male advertisement call and female reciprocal call of *Limnonectes limnocharis*; *Middle*, An enlarged portion of male advertisement call (left) and an enlarged portion of the female reciprocal call (right); *Bottom*, Corresponding mean spectra and sonagram of male advertisement call (left) and female reciprocal call (right) (FFT length: 256; Overlap: 50%; Window: Hamming).

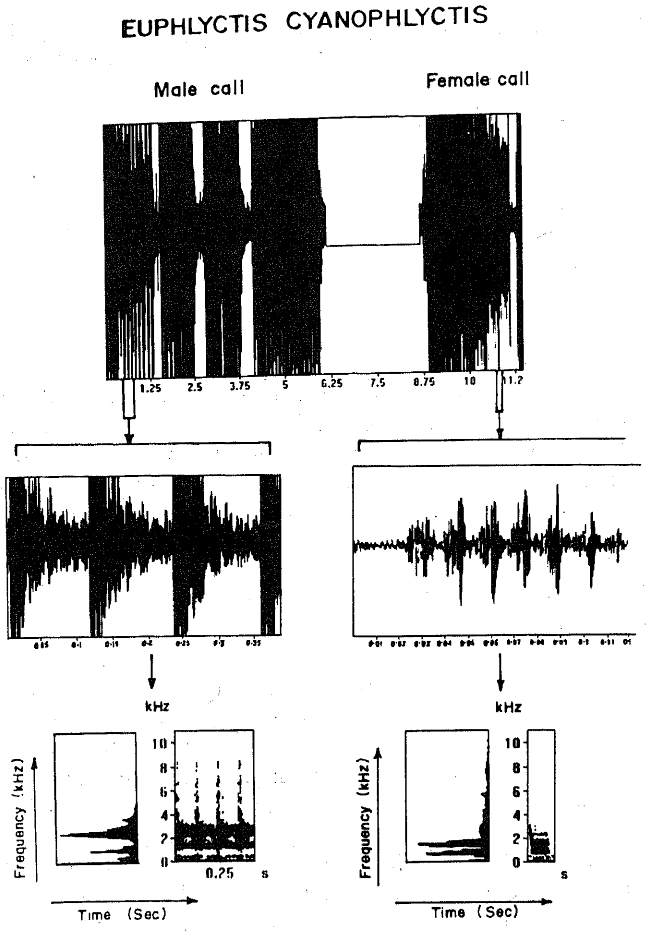

Figure 2. *Top*, Male advertisement call and female reciprocal call of *Euphlyctis cyanophlyctis*; *Middle*, An enlarged portion of male advertisement call (left) and an enlarged portion of female reciprocal call (left); *Bottom*, Corresponding mean spectra and sonagram of male advertisement call (left) and female reciprocal call (right) (FFT length: 256; Overlap: 50%; Window: Hamming).

cloudy or rainy days, with temperature ranging between 23 and 33°C, the call is heard much earlier in comparison to dry hot days around 1630 h. Similarly on cloudy or rainy days, the second male call is heard only 5–10 min after the first male call, whereas on dry hot days the second male begins to call 20–30 min after the first male call.

After the males have advertised for about 30 min, the receptive and responding females which are at times more than one, emerge mostly from under leaf litter. But only one female is seen to climb up and position herself near the first calling male of the evening in the colony. This first calling male occupies the highest perch, positioning itself atleast 80 cm to 1 m above all other calling males of the colony.

This first calling male has a distinct call which sounds like P-A-K and therefore has been termed as the PAK call. It does not change its PAK call pattern even when other calling males approach. It continues to call PAK throughout the evening or till amplexus is reached, while other calling males of the colony produce graded calls depending upon the context which the calls subserve – territorial, aggressive or encounter.

When the distance between the first PAK calling male and the approaching female reduces to about 10 cm, the males which had been calling till then stop advertising. The female then makes a feeble reciprocal call in response to the male PAK call. This feeble call of the female continues at times up to 1 h with several bouts of call. The interval between each bout varies between 2–5 min, with short interval at the beginning and long intervals towards the end of the calling sequence. Only after the female responds to the male advertisement call, the male which had stopped calling on the appearance of the female frog restarts its PAK call but with a higher intensity. The mean intensity before the reciprocal call was 47.62, ± 0.865 dB, while after the reciprocal call it was significantly higher, 58.82, ± 1.459 dB, $n = 93$; $p < 0.05$; *t*-test. In each of the 93 cases without

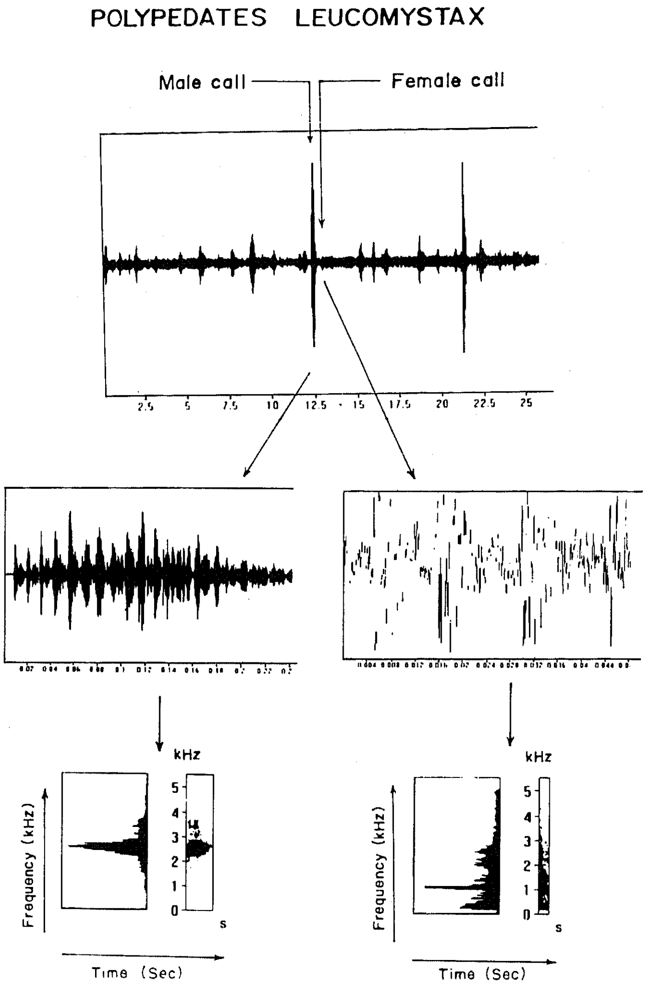

Figure 3. *Top*, Male advertisement call and female reciprocal call of *Polypedates leucomystax*; *Middle*, An enlarged portion of male advertisement call (left) and an enlarged portion of the female reciprocal call (right); *Bottom*, Corresponding mean spectra and sonagram of male advertisement call (left) and female reciprocal call (left) (FFT length: 256; Overlap: 50%; Windows: Hamming)

exception, the intensity of the PAK call was greater than the intensity before. This clearly indicates that the 2 PAK calls are different both statistically as well as functionally. Thus, the advertisement call having higher intensity given after the female call can be classified as 'courtship call'. Concomitantly, all other neighbouring males also start calling at a higher intensity but not as much as the first PAK calling male. More activity is seen amongst the calling males – mostly jumping from one place to another, but all jumps are confined around and across the female which has responded by producing the reciprocal call (Figure 5).

The low pitched feeble reciprocal calls of the females lead to two very significant findings: (1) The female reciprocal call seems to act as a 'catalytic' for the reproductive activity of the breeding colony; (2) Till now the general opinion was that females typically initiate sexual

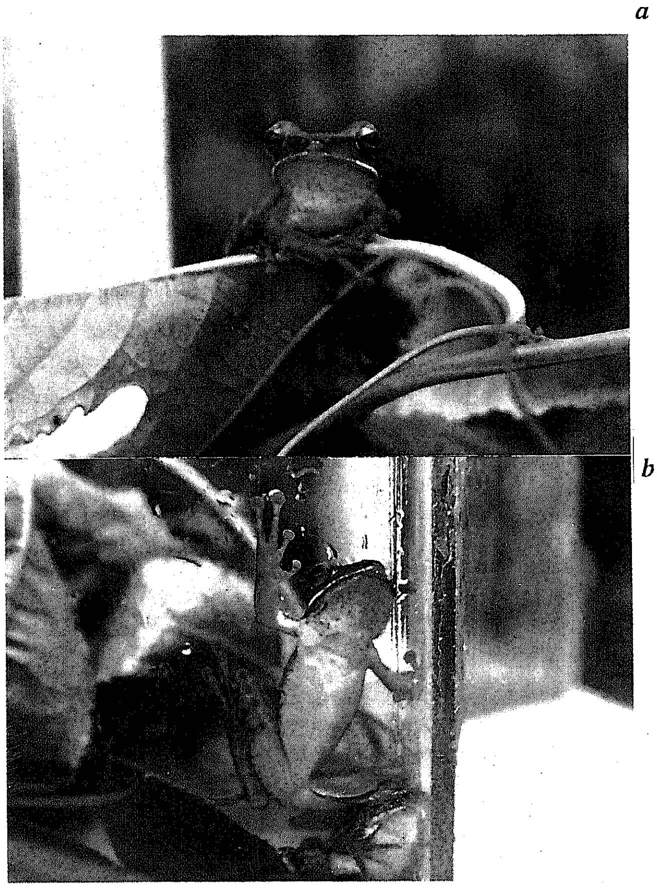

Figure 4 *a, b*. Perching and climbing habit of *Polypedates leucomystax*.

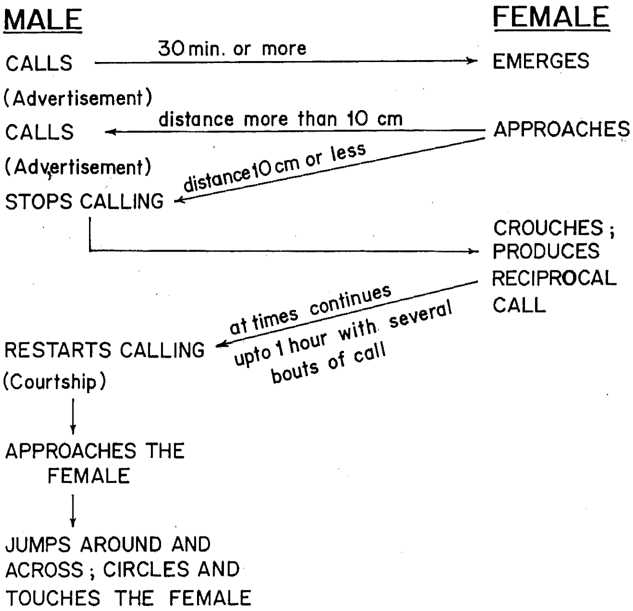
MALE
FEMALE
CALLS (Advertisement)
30 min. or more
EMERGES
CALLS (Advertisement)
distance more than 10 cm
APPROACHES
distance 10 cm or less
STOPS CALLING
CROUCHES; PRODUCES RECIPROCAL CALL
at times continues upto 1 hour with several bouts of call
RESTARTS CALLING (Courtship)
APPROACHES THE FEMALE
JUMPS AROUND AND ACROSS; CIRCLES AND TOUCHES THE FEMALE

Figure 5. Calling sequence and behavioural displays of the advertising male and responding female of *Polypedates leucomystax*.

contact by moving close to or touching a calling male[10,11] and the males indiscriminately try to mate with frogs that move near or touch them[12,13]. The present findings clearly indicate that prior to sexual contact the following should happen sequentially: (i) Distance between the advertising male and the responding female has to be 10 cm or less; (ii) The advertising male which had been calling till then should stop advertising; (iii) The responding female has to respond by producing reciprocal calls; (iv) Males which had stopped calling should restart calling but with a higher intensity – only then sexual contact is established. The establishment of sexual contact in terms of amplexus results after the change from the advertisement call to courtship call and definite moves by both the advertising male and responding female which appear to be distance dependent.

Female mate choice in *P. leucomystax*

During the 3-year study, there were 358 days of field observations which started each day around 1600 h. Out of these 358 observational days, male advertisement calls were heard on 211 days of which the females appeared on 93 days. These data were recorded from 4 different colonies where all the individuals were tagged. The preference of the responding female on all 93 days was always for the first calling male of the colony. This first calling male always occupied the highest perch. At higher perch sites, sound gets less attenuated which is likely to attract more mates[14]. In male *Centrolenella fleischmanni*, calling from elevated sites acquired more mates and suggested this might be due to better sound transmission from elevated as compared to lower call sites[15].

The first calling male is found to be the largest and heaviest amongst all other calling males of the colony. Females are predicted to prefer older males of the colony not only because they may control resources of higher quality, but also because they possess phenotypes that have demonstrated their ability to survive numerous environmental conditions[16]. Benefits obtained by females from their mates can be of two sorts: those affecting the genetic quality of their offspring and those affecting immediate reproductive output. Trivers[17] suggested that all things being equal, females should select older males because they have demonstrated their ability to survive. He also suggested females should select males with faster growth rate because they have demonstrated their resource accrual ability. Larger males are either older or have faster growth rate. To the extent there is a heritable genetic component to growth and survival, these benefits will be passed on to the offspring of both the sexes. The daughters will benefit because of the correlation between female size and the number of eggs per clutch. Sons will have a higher probability of attracting mates if larger. Larger males have been reported to have larger acoustic territory than smaller males. This was probably due to their higher success in fights for calling sites[18] and greater ability to inhibit other males from calling close by.

The first calling male continues to call PAK persistently, does not seem to be disturbed or threatened by the approach of other calling males. It calls at louder intensity after the female reciprocal calls are heard. It is reasonable to assume that males which outsignal competitors enhance the probability of their being noticed by sexually active females[19]. This might be accomplished by employing any of the following strategies: (a) Increase in the intensity of the call[20,21]; (b) Increase in the length of the individual call[22]; (c) Persistent calling throughout the night[23]. The males by employing any of these strategies, regardless of their size, increase the chance of being selected by the sexually active females[23]. In case of *P. leucomystax* the first calling male does not change its call pattern throughout the evening. It continues to call PAK and continues persistently. It calls at a higher intensity after the female reciprocal call. This male seems to employ all 3 strategies. This call is therefore conspicuous, contains more acoustical energy and is indicative of good physical condition. Females which respond to such a male should benefit more[22]. Females of several different anurans prefer calls which are louder, more rapidly repeated, longer and more complex[14,22,24–26]. Males that produce more expensive calls may be fitter than other males in terms of their survival ability and such fitness may be heritable, which is still to be tested.

The females do not appear until the males start advertising and are seen only when they come out and perch themselves next to the first PAK calling male, the distance between the advertising male and the responding female being 10 cm or less. Similar reproductive behavioural results were reported by Ryan[27] in *Physalaemus pustulosus*. Moreover, the conspicuous signaller may be advantageous because such a male may be easier to locate, closer to the female and thus costs of movement incurred by females tend to be minimized[28].

Conclusion

Mating in *P. leucomystax* is non-random like in other anurans[5,29] and is influenced by female mate choice. It chooses males which are larger, heavier, produce louder and lower frequency calls. Sexual selection theory predicts that females may maximize the genetic contribution to future generation through selective mating[30]. The means by which the female anurans differentially recognize fitter males is problematic but atleast in some species, including *P. leucomystax*, fitness is thought to be positively correlated with body size[31–34] and body size may be predicted by parameters of the advertisement calls.

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Stationary prey insures life and moving prey ensures death during the hunting flight of gleaning bats

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While flying, the microchiropteran bats emit brief sounds of high frequencies (15–200 kHz) through the mouth or the nostrils and listen to the echoes reflected from obstacles and prey. The information from the echoes provides the bats an auditory representation of their surroundings with much precision. It is known that sympatrically living echolocating bat species search for prey at different foraging zones. Their audition and structure of the emitted sound signals are adapted to the specific foraging areas and hunting behaviour. A few species of bats use the foraging strategy of gleaning, i.e. capturing prey from the ground and other surfaces including water. Most of the gleaning bats are carnivorous. They use the noise associated with the movement of the prey as the principal cue to detect them on the ground without using echolocation. They use echolocation while capturing prey in the air or on water surface. However, the pattern of echolocation is not similar to the typical pattern shown by other microchiropterans. Normally the hearing sensitivity of the bats is neurologically tuned to the high frequencies of their echolocation calls which vary from species to species. In addition, the ears of gleaning bats are more sensitive to the low frequency noise (5–20 kHz) created by the movement of the prey. Passive listening to the prey-generated noise might be more economical, by collecting available information through the ears, without using echolocation. Such passive method of prey detection is also used by other echolocators like killer whales.

Bats and echolocation

Bats are unique in that they are the only flying mammals, they hang upside down and use echolocation. The microchiropteran bats, smaller and insectivorous, are equipped with an effective system of echolocation for orientation, and to detect and capture prey. The megachiropterans, larger and frugivorous, do not echolocate but have a well-developed visual system for orientation. While flying, the microchiropterans emit a variety of trains of high frequency, brief echolocation sounds consisting of frequency modulated (FM) or constant frequency (CF) signals or a combination of both, with or without harmonics. Such rapid sequences of inaudible (to humans) vocalization are emitted either through the mouth or through the nostrils and bats listen to their echoes reflected from prey and other objects around them. Echolocation enables bats to obtain detailed information about the size, shape, position, range and velocity of flying insects[1]. A typical sequence of echolocation exhibited by pipistrelle bats while detecting and capturing insects has been reported recently[2]. Several field studies showed that different species of microchiropterans forage in distinct habitats that impose very different acoustical constraints on the auditory detection of prey. The type of echolocation signals emitted by each species is associated with its foraging habitat. Neuweiler[3] has distinguished four classes of foraging habitats of bats found in and around Madurai. They are: i) foraging in open spaces, ii) foraging close to or within vegetation, iii) gleaning from leaves and from ground and iv) gleaning from water surfaces. He made an extensive correlation between the preferred foraging habitat and best hearing frequency in echolocating bats. This article is restricted to the foraging behaviour of gleaning bats, emphasizing the methods they use to detect prey.

Gleaning bats

Nearly 30% of about 540 species of Microchiroptera are carnivorous. They hunt for prey by gleaning, i.e. capturing prey on the ground, from tree bark, cliff faces, foliage or from water surface. Table 1 lists a few species belonging to different microchiropteran families that have converged upon the strategy of gleaning[4–20]. Bats like *Antrozous pallidus*[21] and *Megaderma lyra* (personal observations) are captured in small mammal traps, and in the lowest panel of the mist nets at less than a metre above the ground, respectively at their foraging areas. This shows that they spend time on or near the ground possibly searching for prey. Highly manoeuvrable flights and the ability to hover are the essential features for gleaning[22]. Broad, short wings contribute to manoeuvrability, and bats with such wings have often been

Table 1. List of gleaning bats and the surfaces on which they detect and capture prey

Bat species	Surface	Author and reference
Antrozous pallidus; (V)	ground	Bell[4] Fuzessery *et al.*[5]
Cardioderma cor; (M)	ground	Ryan and Tuttle[6]
Macrotus californicus; (P)	ground	Bell[7]
Macroderma gigas; (M)	ground	Kulzer *et al.*[8]
Megaderma lyra; (M)	ground	Fiedler[9], Marimuthu and Neuweiler[10]
	water	Marimuthu *et al.*[11]
Micronycteris hirsuta; (P)	foliage	Belwood and Morris[12]
M. megalotis; (P)	foliage	Belwood and Morris[12]
Myotis blythii; (V)	ground and foliage	Arlettaz[13]
M. emarginatus; (V)	ground	Schumm *et al.*[14]
M. evotis; (V)	ground	Faure and Barclay[15]
M. myotis; (V)	ground	Arlettaz[13]
M. septentrionalis; (V)	bark trellis	Faure *et al.*[16]
Nycteris grandis; (N)	ground	Fenton *et al.*[17]
N. thebaica; (N)	ground	Fenton *et al.*[17]
Otonycteris hemprichi; (V)	ground	Arlettaz *et al.*[18]
Plecotus auritus; (V)	ground	Anderson and Racey[19]
Tonatia sylvicola; (P)	foliage	Belwood and Morris[12]
Trachops cirrhosus; (P)	water	Tuttle and Ryan[20]

M – Megadermatidae, N – Nycteridae, P – Phyllostomidae, V – Vespertilionidae.

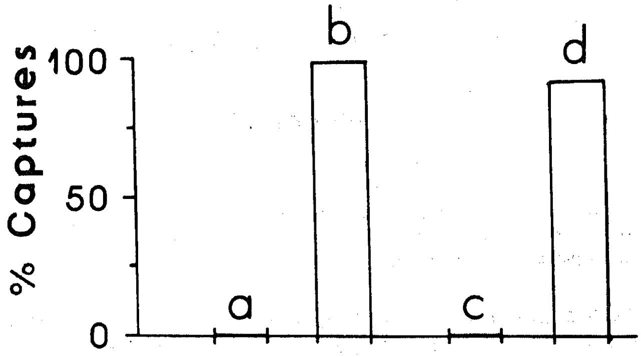

Figure 1. Percentage of captures made by *M. lyra* on live frogs ($n = 104$) when they were stationary (*a*) and moving (*b*) on the sandy floor; and when the freshly killed frogs ($n = 62$) were pulled on the wet glass plate (*c*, without noise) and sandy floor (*d*, with noise); modified from ref. 10.

considered as gleaners. Large pinnae are also characteristic of gleaning bats. An exception is *Euderma maculatum* (Vespertilionidae), a bat with proportionally largest ears among the chiropterans, which does not glean[23]. Most of the gleaners do not use echolocation to detect prey, instead they mainly rely on passive hearing by listening to the rustling noise produced by the movement of the prey.

The Indian false vampire bat *Megaderma lyra*

Megaderma lyra is one of the five species of bats belonging to the family Megadermatidae, distributed in the tropics of Australasia and Africa. It commonly occurs throughout India and lives in caves and unused buildings. Several minutes after sunset, *M. lyra* departs from the day roost and search for ground-dwelling prey such as frogs, mice, geckos and larger insects. while flying low over the ground. By conducting experiments under laboratory conditions, Fiedler[9] demonstrated that *M. lyra* could locate the noise of live mice in complete darkness without using echolocation.

Experiments conducted later at Madurai under semi natural conditions in an outdoor enclosure (7.5 × 3.4 × 3.5 m), showed that *M. lyra* detects and captures live frogs on a sandy floor only when the frogs jump, both under light and darkness[10]. The bats capture freshly killed frogs also when the frog is briskly pulled with a long thread over the floor. However, the bats could not detect stationary frogs, live or dead. In the next set of experiments, it was shown that the bats did not respond when the freshly killed frogs were pulled over a wet glass plate to prevent the noise associated with the movement of the frog. When pulling of the same frogs was continued on the floor, the bats approached and captured them (Figure 1). This clearly shows that *M. lyra* uses the noise of the moving prey as the principal cue for detection. The rustling noise, caused when the prey is moved, has a frequency range of 10–25 kHz. Electrophysiological experiments have shown[24] that the maximum hearing sensitivity of *M. lyra* also falls at a similar frequency range, which is consistent with the idea that bat uses passive hearing to detect prey. It is also seen that *M. lyra* has to touch the prey to decide whether it is palatable or not. For example, when the toad *Bufo melanostictus* jumped, the bat approached and attacked, but returned to roost immediately, leaving the toad on the floor.

In another set of experiments, we found out that *M. lyra* actively echolocated in darkness to detect and capture frogs from the surface of the water[11]. Frogs were available in an artificial pond (4.2 × 2.3 × 0.6 m) and were free moving and distributed themselves throughout the pond. Typically the frogs which stayed at the water surface showed a posture with the dorsal part of the head protruding out of the water. About 30 min after sunset, *M. lyra* began to fly over the surface of the pond. By landing at the edges of the pond intermittently, the bats undertook 'searching flights' in order to detect a frog with head protruding out of the water surface. Then they approached towards the frog with a rapid hovering flight and captured the frog with the mouth which created a sudden splash at the surface of the water. The frog did not show any movement. However, ripples were continuously produced by the movement of frogs staying at other parts of the pond. Figure 2 shows the daily pattern of searching flight activity of *M. lyra*. In most of the observations, the bats succeeded in capturing a frog. However on a few occasions the frogs apparently sensed

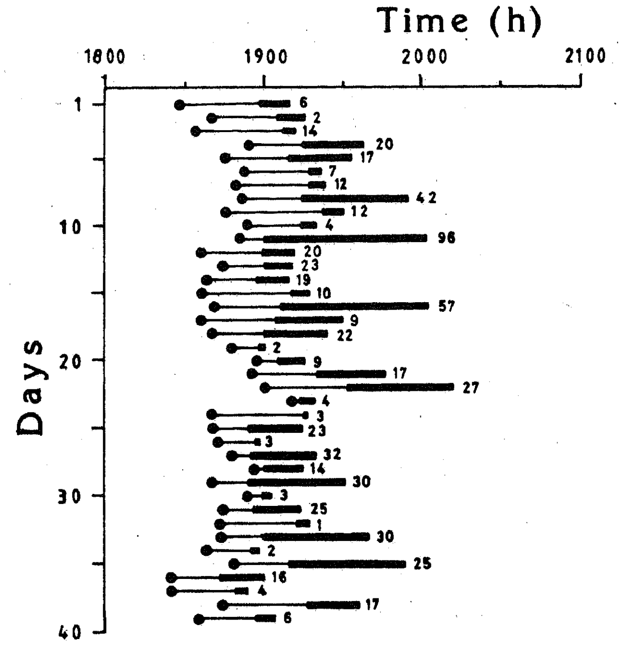

Figure 2. Representative pattern from the noctovision observations on day-to-day changes in the searching flight activity of *M. lyra* to detect and capture a live frog from the surface of the pond in darkness. Black circles indicate the time at which the bat flew out of the roost chamber (180 × 160 × 120 cm) situated at the ceiling of the outdoor enclosure. The horizontal lines following the black circles indicate the duration spent in grooming, stretching one or both wings, etc. before flying towards the pond. The black bands indicate the duration of searching flight (19.9 ± 20.2 min, mean ± SD, range 0.3 to 85.8 min, $n = 89$) to capture a frog at the surface of the pond. The inter-search flight interval was 40.3 ± 27.4 s (range 2 to 123 s, $n = 1041$). The number of search flights is given at the end of black bands and it was 17.1 ± 17.7 (range 1 to 96, $n = 89$).

the danger and dived into the pond as soon as the marauding bats hovered over them. In this situation, the bats either stopped hovering and resumed searching flights or touched the water with their mouths as it was too late to realize the escape of the prey. In this situation the bats stopped flying, spent a short period of time wiping their mouth with the wing membranes and then resumed the searching flights.

While flying at the surface of the water to detect a frog, *M. lyra* used echolocation. A bat detector (Flan 2) and QMC microphone connected separately to the channels of a Lennartz taperecorder were used to record the sounds at a speed of 76 cm/s. The sound analysis showed that *M. lyra* emitted multiharmonic echolocation sounds in all searching flights. Figure 3 shows that the second harmonic is the dominant part of the emitted spectrum (43.6 ± 3.5 kHz, mean, SD, $n = 182$). The sounds emitted about 500 ms before touching the water were too weak for spectral analysis. The final buzz is absent in the flights before capturing the prey. For further details, see Marimuthu *et al.*[11].

It is essential that the head of the frog has to be protruding out of the surface of the water for the bat to detect it. Freshly killed frogs were positioned in three different conditions in the pond: i) head exposed (as in live frogs), ii) the eye balls alone exposed and iii) the entire body submerged just below the water surface. It is clear from Table 2 that *M. lyra* detected and captured all

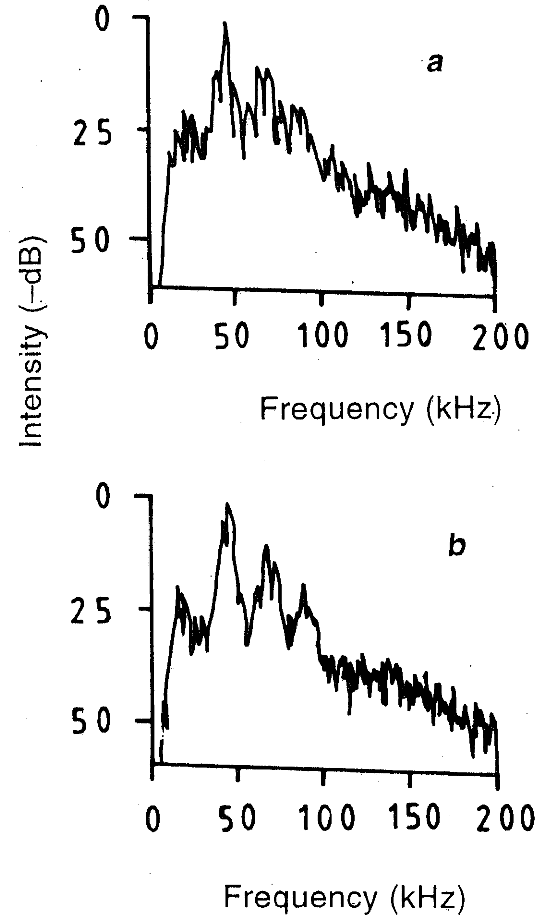

Figure 3. Spectrograms of typical echolocation sounds emitted by *M. lyra* before (*a*) and after (*b*) capturing a frog from the surface of the pond; modified from ref. 11.

Table 2. Searching flights of *M. lyra* to detect and capture a freshly killed frog at the pond in the given period of 20 min. The frog was fixed on a needle-topped and sand-filled ink bottle and the head of the frog adjusted in three positions

Position of the frog at the surface of pond	No. of searching flights (X ± SD)	Time taken to capture a frog (s, X ± SD)	Captures made (%)
Head exposed ($n = 52$)	1.7 ± 0.9 range 1–6	46.5 ± 52.1 range 2–288	100
Eyeballs alone exposed ($n = 39$)	11.6 ± 7.6 range 3–25	439.9 ± 339.9 range 30–1102	33.3
Entire head and body submerged just below water surface ($n = 26$)	23.2 ± 9.5 range 7–39	no capture, but touched water away from frog	Nil

the frogs when their heads were exposed, captured very few frogs (33%) when their eye balls alone were exposed, and that detection was *not* possible when the entire frog was submerged below the water surface. When the positions of the frogs were readjusted with their heads protruding out of the water surface, the bats captured the same frogs with alacrity. The smooth water surface might act as an acoustic mirror from which it is difficult for echolocating bats to receive an echo[1]. The water ripples and the objects protruding from the surface create a kind of texture on the water surface. The echolocation sounds and auditory system of *M. lyra* can detect such fine or coarse surface textures as coloured echoes[1].

Mixed blessing of echolocation

It is evident that echolocation is important in general navigation, for estimation of altitude and detection of obstacles. However, it is not the only method which bats employ to detect and capture prey. Several recent reports show that most of the gleaning bats do not require echolocation cues for prey detection. Plasticity in echolocation is observed in a few species. For example, when adequate illumination is available, *Macrotus californicus* uses vision to locate crickets[7]. Other species like *Antrozous pallidus*[25], *Plecotus auritus*[19] and *Myotis evotis*[15] display both aerial prey capture with echolocation and substrate-gleaning without echolocation. Similarly, *M. lyra* detects and captures prey both on land (using prey generated noise) and in water (using echolocation). Even though gleaning bats echolocate while capturing prey from air or water, their emission of sound sequences is not similar to the typical echolocatory pattern of other microchiropterans. For example, the rapid increase in pulse rate, i.e. feeding buzz emitted by the echolocating bats just before capturing insects, is not found in the echolocation behaviour of gleaners except *Antrozous pallidus*[25], *Myotis emarginatus*[14] and *Nycteris grandis*[17]. In fact the gleaners cease calling about 500 ms before capturing[5,11,15], presumably to avoid alerting the prey. Interestingly *M. lyra*[10], *Cardioderma cor*[6] and *Nycteris grandis*[17] do not respond to the calls of frogs. Similarly *Antrozous pallidus* fails to respond to cricket or katydid calls[5]. In contrast, the phyllostomid bats *Trachops cirrhosus*[20] and *Tonatia sylvicola*[12] respond positively to the advertisement calls of frogs and the mating calls of crickets respectively. *T. cirrhosus* uses both echolocation and passive audition while hunting frogs and is even able to differentiate edible frogs from poisonous toads by the type of their calls. A study[26] on its ear showed that *T. cirrhosus* is sensitive to very low frequencies of less than 5 kHz, which explains how it can hear the calls of frogs.

Thus for bats which use the gleaning strategy of foraging, either on ground or foliage, listening to prey-generated noises sounds might be a more economical way to detect prey than echolocation. Moreover, passive sound localization enables detection of prey even if they are located on 'noisy' substrates which would shield them from echolocation. Hence it is of great interest that the passive method of prey detection is used also by other echolocators like killer whales[27].

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Conflict between parent and offspring in plants: Predictions, processes and evolutionary consequences

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In plants, as in animals, conflict occurs between parents and offspring over a range of issues such as allocation of maternal resources to seeds and number of seeds to be packed in a fruit. We have reviewed theoretical bases and empirical evidences for such conflict in plants and present the predictions, processes and evolutionary consequences of such conflict. We have shown that though 'plants can neither sing nor dance' they do indulge in sibling rivalry, fratricide and kin cooperation as intensely as animals do. For this, maternal parents and offspring have evolved strategies that are subtle, yet as effective as those in animals in upholding their respective interests. We argue that the evolution of a number of plant reproductive features can be profitably viewed as strategies and counter-strategies of the offspring and maternal parent towards ensuring their respective interests. For example, features such as polyembryony, endosperm, multi-carpellary ovaries which had thus far remained poorly explained, appear to have evolved as maternal counter-strategies against offspring-driven sibling rivalry. Thus, these 'highly anthropomorphic, sociobiological hypotheses' which were suggested to be 'best not applied to plants', have indeed been helpful in providing a new framework to view the evolution of plant reproductive strategies.

In humans, the relationship between a mother and her offspring is probably one of the most endearing ones. Across cultures, people have paid obeisance to this sublime relationship. However, in the early 1970s, this view was rudely shattered when Trivers[1] advanced the concept that, more as a rule than an exception, mothers and offspring can have conflicting interests. He argued that such conflict occurs even for the simplest of parental cares, such as suckling; a mother does not feed her offspring indiscriminately while the offspring persists in its demand for its mother's milk. The rationale behind such 'weaning' conflict is the differential fitness accrued to the mother by yielding parental resources, and to the offspring by demanding these resources.

Trivers[1] argued that the maternal parent will be selected to allocate a certain optimal amount of resources to each of her offspring, so as to maximize her inclusive fitness. On the other hand, the offspring will be selected to demand much more than that defined by the mother's optimum to increase its own fitness. Hence there ensues a conflict between parent and offspring over the extent of resource allocation[1,2].

Though Trivers' idea appeared heretical, it provoked widespread interest in the nature of the conflict between parent and offspring in animals. The most dramatic illustration of the conflict is seen in a bird nest where the fledglings demand food from the mother by 'intense begging calls'[3,4]. The mother is coerced to submit to the calls because these intense vocalizations, if persistent, would attract predators risking the entire brood. In a way it may be argued that the fledglings blackmail their mothers to part with parental resources.

The indiscriminate demand for maternal resources by an offspring may be manifested in the form of intense sibling rivalry[1,2,5,6]. In extreme situations, competition among siblings may take a violent form and dominant among the brood kills others to garner all the maternal resources for itself. Brood reduction in boobies and raptors wherein the older chick, invariably kills the later hatched fledglings is one such extreme form of sibling rivalry[7–13].

That sibling rivalry and parent–offspring conflict might also occur in plants was proposed by Ganeshaiah and Uma Shaanker[14] and Uma Shaanker *et al.*[15]. They argued that seeds developing in close physical and temporal proximity in a fruit, can be expected to interact as intensely among themselves as fledglings developing in the nest of a bird (Figure 1 *a*, *b*) and to conflict with the maternal parent over a range of issues. Three issues over which such conflicts can arise are (a) resource allocation to seed[5,15], (b) brood size or seed number packed per fruit[14,15] and (c) seed dormancy or timing of seed germination[15,16]. These conflicts can be explained within the general framework of the theory of parent–offspring conflict proposed by Trivers[1]. Thus whether boobies or brassicas, raptors or rapeseed, it appears that sibling rivalry and parent–offspring conflict may be a common feature of animals and plants alike. In this paper we offer a brief overview of the work conducted at our laboratory on these issues and discuss the predictions, processes and the evolutionary consequences of the conflict between parent and offspring in plants.

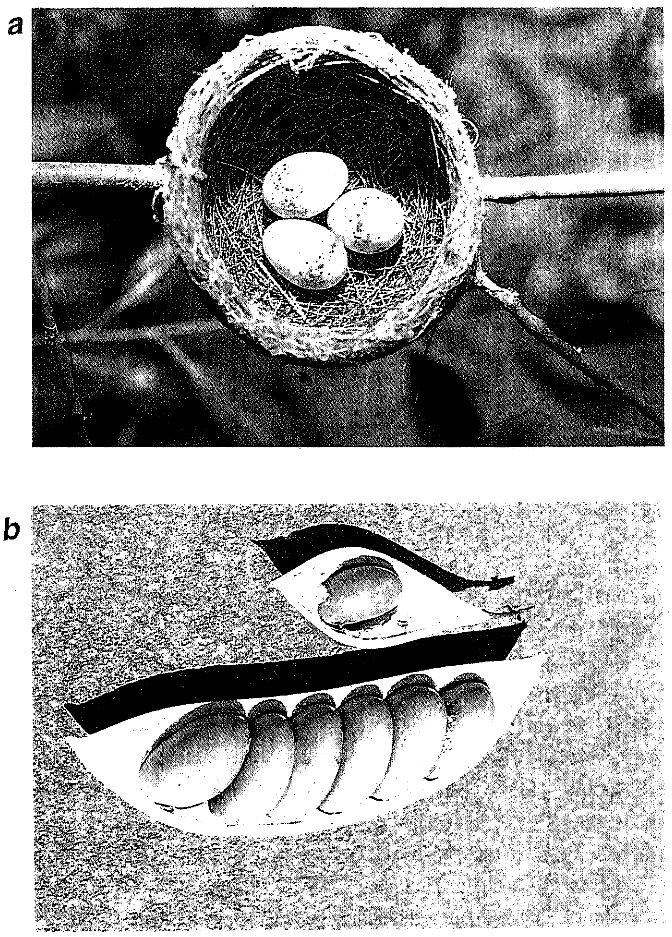

Figure 1. *a*, Clutch of eggs in the nest of a bird and (*b*) clutch of seeds in the pod of the tree, *Castanospermum australe*. Though apparently disparate systems, in both, offspring develop in close physical and temporal proximity with each other as well as with the mother leading to potential for sibling rivalry and parent–offspring conflict. Photo credits: Figure 1 *a*, S. Sridhar; Figure 1 *b*, K. N. Ganeshaiah.

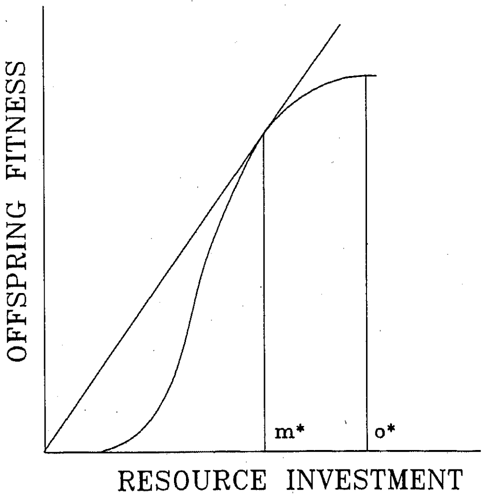

Figure 2. Relation between offspring fitness and resource investment in seed. The region between m^* and o^* represents the zone of conflict (from Uma Shaanker *et al.*[15]).

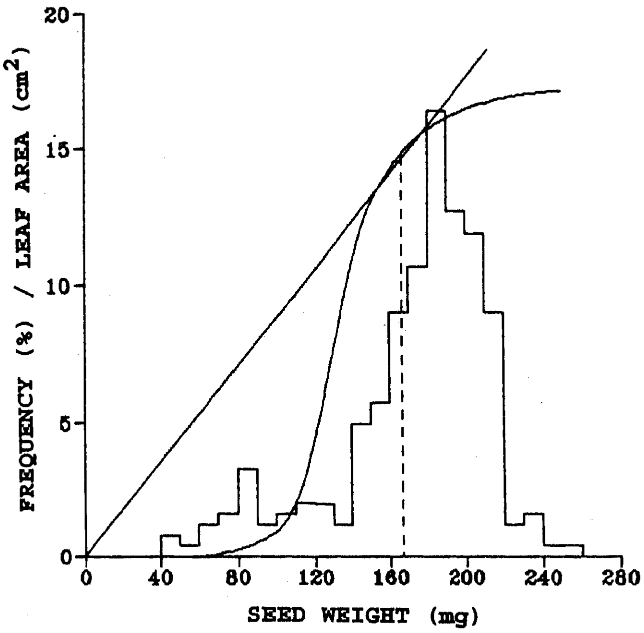

Figure 3. Relation between offspring fitness and seed weight in *Thespesia populnea* and frequency distribution of seed weights. Randomly collected seeds from trees of *Thespesia* were weighed and the frequency distribution plot was developed; offspring fitness was determined by recording the seedling leaf area after 20 days of germination. The optimum seed weight was obtained by drawing a tangent to the fitness curve.

Parent–offspring conflict in plants Predictions and processes

1. Conflict over resource allocation to seeds

Assuming that offspring fitness increases at a decreasing rate with resource investment, Smith and Fretwell[17] argued that the maternal parent would be selected to favour an optimal allocation of resources (m^*) to each of her offspring, such that the marginal returns to her are maximized (Figure 2). However, as Trivers[1] argued, because the offspring by themselves do not invest any resources, they would be selected to demand the maximum amount of investment (o^*) that they can possibly obtain from the maternal parent. This results in a conflict of interest between maternal parent and offspring over the extent of resource allocation to seeds.

Evidence in support of conflict over the extent of resource allocation to seeds has been obtained in *Thespesia populnea* (Malvaceae) at our laboratory (unpublished). In this species, the offspring fitness (as measured by seedling leaf area) increases nonlinearly with seed weight (Figure 3). The optimal level of resource at which the marginal returns to the mother is maximized is 165 mg. If the offspring conform to the maternal interest, then seed size in *T. populnea* should be normally distributed around this optimum. However, the observed seed size distribution was highly skewed (Figure 3) suggesting that the maternal parent and the offspring may have conflicting interests over the extent of resource allocation to seeds.

The indiscriminate demand for maternal resources by an offspring may occur at the expense of the development of its siblings. In fact offspring could be expected to compete intensely among themselves for the maternal resource, leading under extreme conditions to the starvation and death of their siblings. However, the extent of sibling rivalry over resource allocation would be a function of the genetic relatedness among the offspring developing in a fruit[15]. Selection would not favour sibling rivalry among seeds that are genetically related because such rivalry reduces their inclusive fitness. In situations where the seeds are genetically less related, it would pay for a sibling to garner as much of the resource as it can, even at the expense of abortion of its siblings. But, sibling rivalry should theoretically be nonexistent when siblings are completely related. Thus, a greater degree of concordance could be expected between maternal and offspring optima for seeds obtained from self-pollination (high genetic relatedness) than those obtained by cross-pollination (low genetic relatedness).

Evidence in support of this prediction is obtained from the fact that in several species, outbred seeds have a significantly higher seed weight than comparable inbred seeds whose weights more often correspond with the maternal interest[18–21]. In *Vigna unguiculata*, Radha[22] showed that the seed weight of F_0 seeds was significantly influenced by the sibling genetic environment. F_0 seeds developed by pollination with single pollen donors generally resulted in a single peak of seed weight distribution (probably corresponding to the maternal optimum); however when several pollen donors were mixed, the distribution of the weight of seeds sired by any of the donors was characterized by two or more modes and the mean seed weight differed from that obtained under single donor pollination (Table 1). That is, the extent of resource allocation to a seed appeared to be influenced by competing sibling genotypes.

Table 1. Average seed weight (mg) of hybrids in single and mixed cross in *Vigna unguiculata*

Female parent	Cross		Male parents	
		TVX 118-09E	APC 1153	APC 773
Virginia	Single	89.76	113.44	96.52
	Mixed	96.96	91.96	96.64
APC 1078	Single	97.56	112.12	121.24
	Mixed	124.36	123.24	118.60
APC 1016	Single	109.48	137.56	129.68
	Mixed	126.04	128.04	114.20
APC 1034	Single	148.04	129.52	127.36
	Mixed	138.28	148.56	139.44

ANOVA, Female parents $P < 0.01$; Male parents $P < 0.01$

Vigna (cowpea) genotypes were crossed with either pure (single donors) or a mixture of pollen donors, each with a distinct gene marker. The weight of each seed was recorded and the parentage identified at the seedling stage (from Radha[22]).

In *Phaseolus latheroides*, using mixtures of pollen grains derived from 5 and 20 pollen parents, Vasudeva[23] developed pods with decreasing extents of genetic relatedness among the developing siblings. While there was a significant increase in mean seed weight with the number of pollen donors used in the mixture, there was a concomitant increase in seed abortion. The increased rate of abortion was not due to either lack of fertilization of ovules or to lack of resources. Rather, increased seed abortion appeared to be a consequence of intense intra-fruit sibling rivalry for maternal resources with increase in the genetic heterogeneity among siblings.

Manifestation of conflict – war of the hormonal kind?

Conflict over the extent of resource allocation between mothers and offspring is readily apparent in animals. Admittedly, while such overt behaviours cannot be expected in plants, offspring and maternal parents might indulge in subtle but equally effective mechanisms to uphold their respective interests.

One such mechanism could be by signaling through hormones. Plant hormones are small molecular weight, highly diffusible compounds and are known to act at very low concentrations at sites away from where they are synthesized. Consequently, these could be effectively deployed by the offspring and the maternal parent to guard their respective interests. Thus while the offspring could deploy specific plant hormones to 'beg' or 'demand' resources from the mother, the latter could deploy her own hormones to 'quell' the indiscriminate signaling by the offspring for extra resources.

Ravishankar *et al.*[24] examined the hormonal basis of the conflict over resource allocation in plants. They predicted that in a developing seed, the offspring tissues (embryo, endosperm) should be selected to specifically synthesize such hormones that facilitate the uptake of resources, while the maternal tissues (seed coat, pericarp) should be selected to produce hormones that restrict resource uptake. Analysing both the genetic and physiological data on a number of plant systems, they provided evidence in support of these predictions. They showed that hormones such as gibberellic acid (GA) and indole acetic acid (IAA), that facilitate the active mobilization of photoassimilates from the maternal sporophyte into the developing seeds, are exclusively synthesized by the offspring tissue (embryo/endosperm). On the other hand, abscisic acid (ABA), which restricts the mobilization of resources into the seed, is exclusively synthesized by the maternal tissues of the seed (seed coat, pericarp, etc.) and is deployed at a time coinciding with that of IAA or GA production in the seed (Figure 4). In other words, even as the offspring deploy hormones to draw resources from the mother, the latter deploys its own arsenal in the form of abscisic acid to negate the indiscriminate demands made by the offspring. Thus, there might be a dynamic conflict between the mother and offspring over the extent of resource mobilization into the seeds, through the deployment of specific hormones.

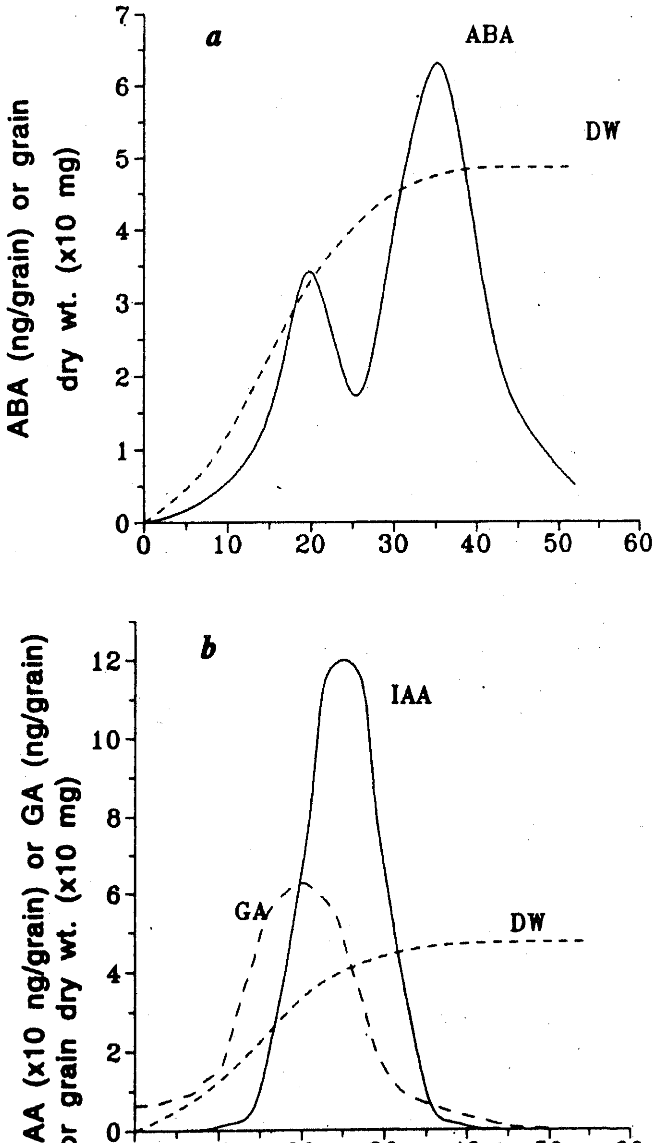

Figure 4. Deployment of hormonal strategies in the conflict over resource allocation between maternal parent and offspring during seed development. Temporal pattern of accumulation of (*a*) abscisic acid (ABA) and (*b*) auxins (IAA) and gibberellic acid (GA) and grain dry weight (DW). Notice the two peaks of accumulation of ABA; while the second peak is reported to impart desiccation tolerance to the embryo, the significance of the first peak is not known. It is proposed that the first peak may function as a maternal strategy to restrain the indiscriminate demands of the offspring for the maternal resources (from Ravishankar *et al.*[24]).

2. Conflict over brood size

In plants, several components of offspring fitness such as dispersal efficiency[14,15], escape from predation[25] and post-dispersal seedling survival[26,27] decrease with increase in the number of seeds packed per fruit (brood size). For instance, in species where the entire fruit is dispersed as a unit through wind, water or animals, the dispersal efficiency of the fruits decreases with the seediness of the fruits[14,15,28–34]. Furthermore, seeds in a large brood are more likely to be preyed upon than those in a small brood[25]. Ganeshaiah and Uma Shaanker[14] and Uma Shaanker *et al.*[15] argued that under these conditions, selection would favour each sibling in a fruit to indulge in fratricide and kill others to become the lone survivor in order to enhance its own individual fitness. However such fratricidal behaviour among the offspring can directly conflict with the interest of the maternal parent. The maternal parent would be selected to optimize her brood size as a trade-off between the benefits (dispersal advantage, etc.) and the cost of packing seeds in fruits[15]. Since the offspring does not incur any packing cost, it will always be selected to favour a brood size smaller than that the mother is selected to favour.

Based on inclusive fitness models, Uma Shaanker *et al.*[15] analysed the conditions under which brood reduction will be favoured by the offspring or by the maternal parent. They showed that for a relatively low ratio of benefit (increased dispersal advantage due to brood reduction) to cost of killing sibs (loss of inclusive fitness due to brood reduction), the offspring would favour brood reduction while the maternal parent would not (Figure 5).

Thus, under conditions where offspring fitness is a decreasing function of the number of seeds packed in a fruit, Uma Shaanker *et al.*[15] predicted that selection would favour fratricide or sibling rivalry-driven seed abortion compared to situations where offspring fitness is unaffected by changes in brood size. Indeed in a study of over 200 species, Uma Shaanker *et al.*[15] found that seed abortion was significantly higher in species whose fruits were dispersed by wind, water or animals (where dispersal efficiency decreases with brood size) compared to those where the seeds were passively dispersed (where dispersal efficiency is independent of the size).

Manifestation of conflict – nature green but thorny and poisonous

In recent years, certain unique mechanisms of intra-fruit sibling rivalry leading to seed abortion have been

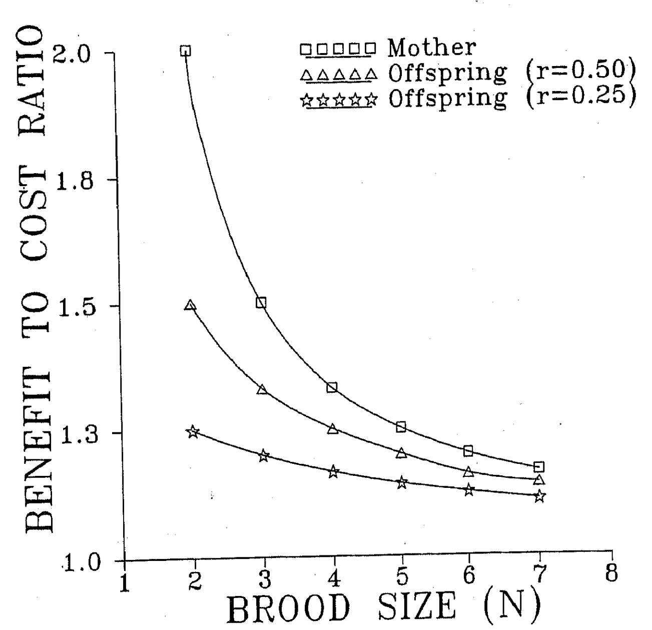

Figure 5. The relative ratio of benefit (B) to cost (C) required to favour brood reduction by mother and offspring. Note that for all values of brood size (ovule number per ovary), offspring favour brood reduction for relatively lower B/C ratios than the mother. Also note that the ratio required to favour brood reduction by the offspring decreases with decrease in the coefficient of relatedness among the siblings (modified from Uma Shaanker *et al.*[15]).

reported in plants[14,36,38–41]. These studies suggest that though plants may not match the adage 'nature red in tooth and claw', they might represent 'nature green but thorny and poisonous'.

Sibling rivalry in Dalbergia sissoo

In the wind-dispersed tree, *Dalbergia sissoo*, predominantly only one of the four or five ovules in an ovary develops to maturity[14]. Ganeshaiah and Uma Shaanker[14] proposed that seed abortion in *Dalbergia* could be due to intra-fruit sibling rivalry to enhance the surviving seed's dispersal efficiency. The abortion of the rest of the 3 or 4 seeds occurs within a week after fertilization and is not attributable to either lack of pollination or resource limitation[14,15,35–37]. Abortion is mostly restricted to ovules at the base of the pod (distal to the stigmatic end). In the presence of the dominant stigmatic embryo, the uptake of labelled sucrose by the basal embryos is significantly inhibited. However, when their dominance was removed (by excising the dominant embryos), the uptake of labelled sucrose by the basal embryos equalled those of the dominant embryos. In fact, when the dominant stigmatic embryo is surgically excised, the ovules at the base develop normally. In other words, abortion of the basal embryos is probably a consequence of their starvation by the dominant embryos developing at the stigmatic end.

Mohan Raju *et al.*[36] showed that dominance may be exerted by the production of a chemical by the stigmatic embryo. Extracts and diffusates of the dominant embryos significantly inhibited the uptake of labelled sucrose by subject (fertilized) embryos while those of control tissues such as fruit coat (maternal tissue) and unfertilized ovules did not. In other words, abortion of basal embryos appeared to be actively mediated by sibling rivalry (fratricide) and not due to any maternal intervention (infanticide). These results corroborate the prediction that seed abortion in fruits is favoured more by interests of the offspring than that of the mother. While the chemical nature of the diffusate causing the abortion is not clear, it is conjectured that it may be an indole compound, very likely the 'death hormone', 3-chloro-indole acetic acid[36]. A similar mechanism of seed abortion has been reported in the animal-dispersed species, *Syzygium cuminii*, where invariably only one out of 25 to 30 ovules develops to maturity[38–41]

Based on these results, a general model explaining seed abortion in plants is proposed. According to this model, temporal differences in fertilization set in a dominance hierarchy among the ovules. The early fertilized, dominant embryos thus formed, gain a head start in the production of certain 'indole' compounds. These compounds not only further facilitate the build up of dominance due to a feedback flow of resources to the dominant ovules, but also starve the remaining later fertilized ovules of resources, thereby either directly or indirectly leading to their abortion[41]

Sibling rivalry-driven brood reduction – function of genetic relatedness among sibs?

Based on inclusive fitness models, Uma Shaanker *et al.*[15] showed that the benefit-to-cost ratio required to favour brood reduction by offspring decreases with a decrease in genetic relatedness among the siblings (Figure 5). Consequently, the intensity of conflict between the mother and offspring increases with a decrease in the genetic relatedness among the siblings. For relatively small benefits due to brood reduction, an offspring would be more selected to favour killing its siblings when they are half sibs ($r = 0.25$) than when they are full sibs ($r = 0.50$) (Figure 5). Thus, they predicted that sibling rivalry-driven brood reduction should be more intense in outbred compared to inbred conditions.

Several lines of evidence support this prediction, albeit indirectly. In pigeon pea, intra-fruit seed abortion was positively correlated with the extent of outcrossing[42]. In *Epilobium*, congeneric species that were outbred had a higher degree of seed abortion than those that were inbred[43,44]. In *Phaseolus latheroides*, as mentioned earlier, Vasudeva[23] showed that the extent of seed abortion increased dramatically with an increase in the number of pollen donors used for pollination.

Evolutionary consequences

If rivalry among siblings is in conflict with the interests of the mother, selection can be expected to favour counter-strategies in the maternal parent that either reduce sibling rivalry or make good the loss of fitness incurred to her as a result of rivalry. Here we examine briefly a few features of plant reproduction that might be argued to have evolved as maternal counter-strategies against the conflicting interests of the offspring.

Evolution of polyembryony – maternal counter-strategy against offspring-driven brood reduction?

Polyembryony, the differentiation and development of additional embryos from the tissues of the embryo sac, is a widespread phenomenon in angiosperms. An interesting feature, however, is that in over 78 per cent of species, the additional embryos emerge from the maternal tissues of the embryo sac (e.g. nucellus and integument) and in only about 22 per cent of species, they emerge from the cleavage of the sexual embryo or endosperm[45]. The evolutionary significance of this feature has largely remained unaddressed.

Ganeshaiah *et al.*[45] and Uma Shaanker and Ganeshaiah[46] suggested that polyembryony may be viewed as a possible maternal strategy of making good the fitness lost through offspring-driven brood reduction. Based on both genetic and inclusive fitness models, they showed that the maternal parent should favour the production of additional embryos for relatively small gains of producing them while the offspring do not (Figure 6). On the contrary, for relatively small benefits, the offspring favour brood reduction, while the mother does not (Figure 5). Thus, it is clear that in both situations, there is a conflict of interest between the maternal parent and the offspring over brood size. Ganeshaiah *et al.*[45] argued that the evolution of polyembryony may hence represent a maternal strategy of making good the fitness lost due to offspring-induced brood reduction. Accordingly, they predicted that polyembryony should be more frequent in species that exhibit high rates of intra-fruit seed abortion than in those where seed abortion is less intense. In support of this prediction, they found that polyembryony was more frequent than expected in species where the entire fruit is dispersed as a unit through wind, water or animals (characterized by a high degree of seed abortion) than in species where the seeds are passively dispersed (characterized by lower rates of seed abortion).

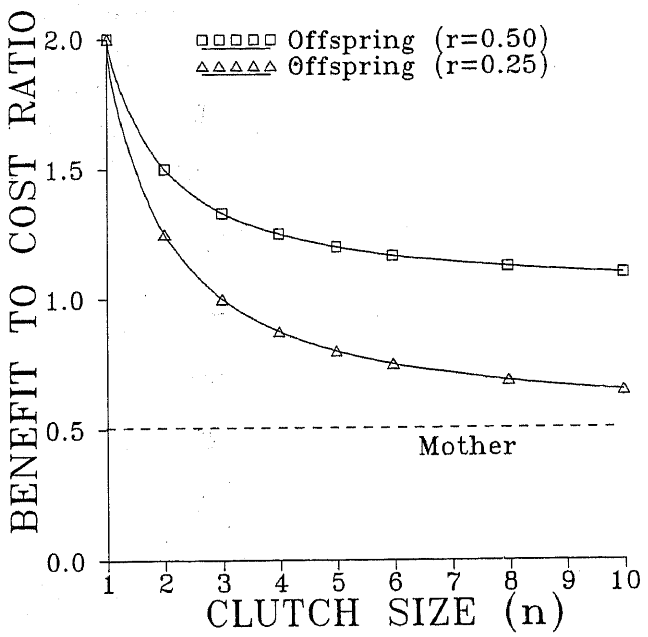

Figure 6. The relative ratio of benefit (B) to cost (C) required to favour polyembryony by the mother and offspring. Note that for all values of clutch size (ovule number per ovary), the mother favours the production of polyembryos for a lower B/C ratio than the offspring. Further, the ratio required to favour polyembryony by the offspring increases with increase in the genetic relatedness among the siblings (from Ganeshaiah *et al.*[45]).

Furthermore, they also found a significant positive correlation between the number of polyembryos produced and the extent of seed abortion across species of *Citrus*.

Evolution of endosperm – an enigma resolved?

One of the most perplexing features of the angiosperm seed is the triploid storage tissue, the endosperm. With its two doses of maternal and one of paternal chromosomes, the evolution of endosperm has remained an enigma. Functionally, it draws resources from the maternal sporophyte and serves as a repository until the embryo is capable of using it. In most species, the endosperm degenerates as the seed matures; in others it is persistent and offers resources to the developing seedling. It has been argued that the endosperm, with its unique genetic composition might represent (a) paternal interest by increasing the genetic relatedness of the nourishing tissue to its own embryo such that there will be a greater allocation of resources to its own embryo[47] or (b) maternal interest by subduing the indiscriminate demand of resources by the offspring[48]. According to the latter argument, the endosperm as a nourishing tissue will be less biased in discriminating among the embryos than if embryos themselves were to function as nourishing tissue. In other words, it is argued that endosperm

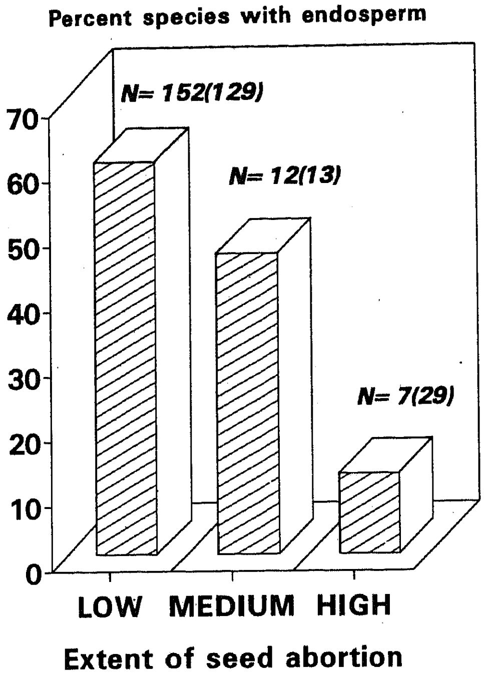

Figure 7. Relation between extent of seed abortion and presence of endosperm. Species ($N = 335$) with varying extent of seed abortion were analysed for the presence or absence of well-developed endosperm (contingency chi-square analysis, $\chi^2 = 42.53$, $p < 0.005$, $df = 2$). Species with high extent of seed abortion had endosperm less frequently than expected while those with less seed abortion had endosperm more often than expected. Values in the figure refer to species with endosperm; those in parentheses indicate the expected frequency (from Uma Shaanker *et al.*[49]).

might represent a maternal strategy to reduce intra-fruit sibling rivalry for maternal resources.

Accordingly, Uma Shaanker *et al.*[49] predicted that the endosperm should be more frequently found in multi-ovuled species (where there is a greater potential for sibling rivalry) than in uni-ovuled species. Furthermore, in species with well-developed endosperm, there should be less seed abortion (brood reduction) than in species with less well-developed endosperm. Analysing the association between the presence or absence of endosperm in 1131 species, they found that both these predictions were upheld. Well-developed endosperm was present more frequently than expected in multi-ovuled species than in uni-ovuled species. Furthermore, in species with a high degree of seed abortion, endosperm was less frequent than expected, compared to those in which seed abortion was less (Figure 7). Thus it appears that the evolution of the triploid endosperm might represent a maternal strategy of maintaining a more equitable allocation of resources among the siblings and thus reducing sibling-driven seed abortion or brood reduction.

Other features

A number of other flower and seed features might similarly be viewed to reflect a maternal counter-strategy in subduing the conflicting interest of the offspring. The evolution of the hard seed coat or glumes, apart from their well recognized role of imparting protection to the embryos might also function to physiologically limit the indiscriminate mobilization of resources by the offspring[15]. The evolution of septa or locules between ovules might serve to insulate the siblings from intra-fruit sibling rivalry[15]. For example, in *Thespesia populnea*, where the ovary is pentacarpellary with three ovules in each carpel, seed abortion was found to be distributed across the carpels; mature fruits invariably had at least one seed in each carpel. Uma Shaanker *et al.*[49] showed that the development of endosperm and of locules might occur in a mutually exclusive manner. They found that well-developed endosperm occurs less frequently present than expected in species with locules than in those without locules.

Conclusions

When the concept of sibling rivalry and parent–offspring conflict was extended to plants, Wiens *et al.*[50] remarked that 'these highly anthropomorphic, sociobiological hypotheses are best not applied to plants'. However studies over the last decade have proven otherwise[51,52]. Work in our laboratory and elsewhere have shown that as much as animals, plants too exhibit sibling rivalry and parent–offspring conflict over issues ranging from the maternal resource allocation to seeds to seed packing in fruits. Apart from providing a strong theoretical framework to explain the conditions under which the maternal parent and offspring have conflicting interests, our studies have provided empirical evidence in support of sibling rivalry and parent–offspring conflict and have unravelled the underlying proximate mechanisms through which the conflicting interests are manifested. Though the interactions may not be as apparent and dramatic as that in animals, plants have evolved equally effective, but subtle mechanisms to uphold the respective interests of the maternal parent and offspring. One of the major implications of our studies is that they have provided a new framework to view the evolution of a number of plant reproductive features, such as polyembryony, endosperm and locules, which hitherto had largely remained unaddressed.

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Alternations of sexes in a deciduous tree: temporal dioecy in *Bridelia retusa*

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Bridelia retusa **(Euphorbiaceae), a monoecious tree species at Bhimashankar, Maharashtra, was found to exhibit temporal dioecy – a system of sex expression in which there is little or no overlap between the production of male and female flowers within an individual tree during the flowering season. There was variation between individuals and between populations in the number of sex alternations. Some individuals that were phenotypically more male were functionally more female relative to the gender of other individuals in the population. The possible advantages of this breeding system are discussed and experiments to confirm these postulations are suggested.**

ANGIOSPERMS present a bewildering array of sexual systems ranging from hermaphroditism to dioecy. Hermaphrodites either have perfect hermaphroditic flowers or are monoecious with separate male and female flowers on the same individual. In dioecious species, male and female flowers occur in separate individuals. Between hermaphroditism and dioecy are sexual systems incorporating such sex expressions as gynomonoecy (coexistence of hermaphroditic and female flowers on a single plant), andromonoecy (coexistence of hermaphroditic and male flowers on a single plant), gynodioecy (coexistence of hermaphrodites and female individuals in a population), and androdioecy (coexistence of hermaphrodites and male individuals in a population).

Although these sexual systems were recognized and named by Linnaeus and Darwin, the selective forces responsible for their origin, evolution and maintenance are still unresolved[1–4]. Avoidance of inbreeding, optimal allocation of reproductive effort between male and female function, sexual selection, and the interaction of these forces with ecological factors have been proposed as explanations for the variety of sexual systems in plants[5–8]. The understanding of the evolution of sexual systems is further complicated by the fact that many species also possess traits such as genetic self-incompatibility mechanisms, heterostyly, cleistogamy, and dichogamy. Traits such as dichogamy, in which there is a temporal separation of male and female phases within a flower, effectively convert a morphologically perfect-flowered hermaphroditic individual into a plant bearing functionally unisexual flowers at any given time.

The interplay between morphology and functionality is of considerable interest in the evolution of sexual systems such as temporal dioecy. The term temporal dioecy was coined by Cruden and Hermann-Parker[9] to describe a breeding system in which there is temporal alternation of male and female function within an individual during a single flowering season. It describes a flowering pattern in which the staminate and pistillate phases of an individual are so distributed that there is little or no overlap between the male and female phases of an individual hermaphroditic plant at any given time. In plants with perfect flowers, this can be achieved by synchronous dichogamy via successive episodes of either synchronized protandry or protogyny[10]. In monoecious plants, it can be arrived at by second-order dichogamy[11] in which the production of male and female flowers alternates through time within an individual plant.

Although temporal dioecy was first mentioned by Darwin[12], it has usually been thought of as rare and anomalous and has been neglected as a breeding system[13]. There has been almost no theoretical investigation of the conditions responsible for the evolution of temporal dioecy relative to true dioecy[14]. Temporal dioecy can also occur with variable frequency among individuals of a population, with some individuals exhibiting temporal dioecy while others do not[14]. There is also intra-population variation in the number of sex alternations and in the success of the same sexual phase at different times during a single flowering period[13,15].

In this paper we report the occurrence of temporal dioecy in *Bridelia retusa* (Euphorbiaceae) and record variation in the frequency of sex alternations among individuals in two sample populations. We speculate on the advantages of this breeding system and we suggest experimentation to confirm these postulations.

Methods

Bridelia retusa is a monoecious, deciduous tree. We sampled the flowering phenology of 23 trees (13 in a forest patch named Adhal – Population 1; 10 in a forest patch named Hindola – Population 2) at the Bhimashankar Wildlife Sanctuary, Pune District, Maharashtra. We collected a minimum of two flowering twigs from each of these trees on the following dates: 22 April, 28 April, 4 May, and 26 May 1996 (henceforth referred to as Times I–IV). From these samples, for each tree, we counted the number of open male and open female flowers. We dissected flower buds and noted the presence of male and female buds and their relative sizes. We recorded the presence and relative sizes of fruit. For each sample day, we calculated the phenotypic gender (P_{id}) of each tree by the formula $P_{id} = D_{id}/(D_{id} + L_{id})$, where D_{id} is the total number of open female flowers in the sample from the ith tree on day 'd', and L_{id} is the total number of open male flowers in the sample from the ith tree on day 'd'[16]. This measure can vary from 0 (male phase only) to 1 (female phase only). For each sample day, for each tree, we also calculated S_{id} – the standardized phenotypic gender following Lloyd and Bawa[17]. S_{id} weights investment in male function according to the relative availability of pollen and ovules in the entire population.

$$S_{id} = \frac{D_{id}}{D_{id} + (L_{id} E_{id})}.$$

E_{id} is an equivalence factor and is given by $E_{id} = (\sum(D_{id}/L_{id}))/i$. It is the average ratio of female to male flowers within the samples for all trees sampled on a given day. S_{id} also varies from 0 (male phase only) to 1 (female phase only).

Table 1. Phenotypic gender (P_{id}) and standardized phenotypic gender (S_{id}) of individuals of *Bridelia retusa*

Tree no.	I	II	III	Time I	II	III
		P_{id}			S_{id}	
Population 1 (Adhal site)						
1	0.35	0.02	0.00	0.13	0.94	0.00
2	0.88	–	0.25	0.66	–	0.81
3	–	0.00	0.00	–	0.00	0.00
4	1.00	0.00	–	1.00	0.00	–
5	1.00	0.00	0.00	1.00	0.00	0.00
6	0.00	0.00	–	0.00	0.00	–
7	1.00	–	0.00	1.00	–	0.00
8	0.94	0.00	0.00	0.82	0.00	0.00
9	1.00	1.00	0.00	1.00	1.00	0.00
10	0.00	0.00	0.00	0.00	0.00	0.00
11	0.50	–	0.00	0.22	–	0.00
12	0.89	–	0.40	0.69	–	0.90
13	0.88	1.00	0.00	0.66	1.00	0.00
Population 2 (Hindola site)						
1	0.08	1.00	0.00	0.86	1.00	0.00
2	0.00	0.00	0.63	0.00	0.00	0.62
3	0.00	0.00	–	0.00	0.00	–
4	0.00	1.00	0.00	0.00	1.00	0.00
5	0.00	1.00	0.00	0.00	1.00	0.00
6	0.00	1.00	0.00	0.00	1.00	0.00
7	0.03	0.00	0.00	0.65	0.00	0.00
8	0.00	0.00	0.00	0.00	0.00	0.00
9	0.03	0.00	0.00	0.70	0.00	0.00
10	0.00	0.00	0.90	0.00	0.00	0.90

Times I, II and III are 22.4.96, 28.4.96 and 4.5.96 respectively. Blanks indicate samples with no open flowers of either sex. Values of both P_{id} and S_{id} range from 0 (male phase only) to 1 (female phase only).

Results

The flowering and fruiting season of *Bridelia retusa* had already commenced when we started collecting samples. By 26 May, 1996 (Time IV), flowering had nearly ended with samples from only 5 trees in Population 2 bearing open male flowers (2–6 flowers) which were considerably smaller than the male flowers of earlier cycles. At Time IV, samples from only 2 trees (one in each population) had a few, small male buds. There was no evidence that any further buds of either sex would arise on any of the sampled individuals. We, therefore, calculated phenotypic gender only for Times I, II and III. The pattern of change in the phenotypic gender varied among individuals and between the two populations (Table 1). In Population 1, the change was mostly from female to male with the exception of Tree # 7 which remained all-male. This tree did not produce any open female flowers, female buds or fruits throughout the observation period. In Population 2, there were complete reversals from male to female to male phases (4/10 individuals) within the short duration of 12 days (22 April–4 May), as well as male to female alternations (2/10 individuals). Two plants were recorded with only open male flowers during the sample period, although they did produce fruit. There were no all-male trees in Population 2.

The standardized phenotypic gender measures indicate that in Population 1, two trees which were phenotypically mostly male were 'functionally' more female when compared to the gender of the population at that time (Tree # 1 at Time II, and Tree # 2 at Time III; Table 1). Similarly, Tree # 1, 7, and 9 of Population 2 were 'functionally' female when phenotypically mostly male at Time I (Table 1).

Since our sampling of the flowering phenology did not begin at the onset of the flowering season, there were already fruit present in some samples taken at Time I. Also, after Time III, there was a 22-day interval during which no samples were collected. Based on the presence and size of fruit in the samples collected at Time I and on the sex of the buds available in the samples at Time III, we inferred the presence of a female or male phase prior to Time I and after Time III (Table 2). Based on these projections, there appear to be mostly female to male transitions in Population 1 but several cycles of transitions in Population 2.

Discussion

Unlike animals which can usually respond to unfavourable environmental conditions or unfavourable sex ratios by moving between environments or populations, plants are sessile, and can only respond to adverse situations by complex patterns of growth and reproduction. In animals, low or no mobility as in sessile forms, has been advanced as an important factor in the evolution of simultaneous hermaphroditism in which both sexes are simultaneously expressed and could self-fertilize if necessary[18]. The size-advantage hypothesis has been usually invoked for explaining sequential hermaphroditism in animals, in which individuals start out as either male or female but switch sexes as they become larger and/or older[18]. These animal-based ideas were the origin of theories for the evolution of hermaphroditism versus dioecy in plants wherein the law of diminishing returns would influence a plant's decision to invest reproductive effort in combined or separate sexes[5]. Avoidance of self-fertilization, optimal allocation of reproductive effort via maleness or femaleness, local mate competition, and sexual selection, coupled with environmental conditions conducive for male or female reproduction are some of the current explanations for the variety of sexual systems found in plants[6, 19–21].

Table 2. Alternations of sexes in *Bridelia retusa* individuals based on phenotypic gender, fruit and bud sex

Population 1 (Adhal site) (n = 13) Alternation	 Number of individuals
F → M	10
M → F	1
F → M → F	1
All M	1
Population 2 (Hindola site) (n = 9*) Alternation	 Number of individuals
F → M	2
M → F	1
F → M → F	1
M → F → M	2
F → M → F → M	2
M → F → M → F	1

Alternations based on phenotypic gender at Times I, II, and III and on presence of fruit at Time I (indicative of at least one female phase prior to Time I), and of bud sex at Time III (indicative of identity of sex phase after Time III).

* In case of Tree # 3, there were no fruit prior to Time I, no female flowers at Times I–III, but mature fruit at Time III. The possible sequence of sex alternations in this tree could not be determined, resulting in $n = 9$ instead of $n = 10$ in Population 2 in this case.

Temporal dioecy is reported to occur in 122 genera of at least 37 angiopserm families and is believed to be strongly associated with monoecy and self-compatibility[13]. Temporal dioecy is considered to be adaptive because it can maximize fitness by minimizing geitonogamy and maximizing outcrossing[13]. After investigating a large sample of hermaphroditic and monoecious angiosperms, Bertin[22], however, argued that self-fertility may be independent of sexual system; for instance, dichogamy is equally common among self-compatible and self-incompatible species. Whether *B. retusa* is self-compatible remains to be determined.

Temporal dioecy has also been suggested as a bet-hedging strategy in plants (sensu Stearns[23]) by several workers[15,16,24]. By alternating sexes throughout the flowering season, it is believed that temporally dioecious plants can counteract the negative effects of factors such as local mate competition, and can optimize reproductive effort through either male or female function given the unpredictable nature of the sexual state of neighbouring individuals, i.e. the mating environment of flowers (sensu Brunet and Charlesworth[25]), and thereby the unpredictability of reproductive success. From this perspective, it would also be interesting to determine whether individual plants are plastic or fixed in the duration and number of sex alternations over successsive flowering seasons, and in relation to their neighbours. For example, in *Atriplex canescens*, some clones have fixed gender expressions while in others, gender varies, and the magnitude and direction of sex change is both genetically and environmentally determined[26].

In our limited investigation of the flowering of *Bridelia retusa*, we found evidence for several alternations of sexes in some individuals and at least one individual which was all-male. We do not know if the sequence and durations of sex alternations observed in these two populations will be repeated during subsequent flowering seasons, and whether the all-male tree will continue to remain unisexual. It is possible that this year, the all-male tree was merely exhibiting an extreme in a continuum of intra-tree sex ratios possible within a monoecious species[27]. Yet, recent studies have revealed the presence of stable sexual polymorphisms within populations of plant species which may include a combination of males, females and hermaphrodites some of which are temporally dioecious[14,28]. Continued monitoring of marked *Bridelia* individuals over several flowering seasons will be necessary to determine the stability of sex expression in this species.

We also found that some plants which were phenotypically more male were 'functionally' more female relative to the gender of other individuals in the population. The phenotypic gender of other individuals in the population can cause the uncoupling of phenotypic and functional gender of an individual[29]. In the temporally dioecious *Aralia hispida*, Thomson and Barrett[15] found that the reproductive success of a ramet through pollen was negatively correlated with the degree of flowering synchrony between the ramet and the population.

In this study we have observed differences between two *Bridelia* populations, albeit on a short time scale. Population 2 appeared to have more types of sex alternations than the other. It is possible that the relation between local environment and sex expression also needs to be investigated. Consistent differences between *Bridelia* populations at the two sites over several flowering seasons may be indicative of an environmental effect. Directional sex change in plants is known to be a function of environmental conditions such as soil fertility where greater fertility causes more femaleness or where higher temperatures cause maleness[8,30,31]. Females appear to be produced when stress is lacking.

Can the *Bridelia retusa* system be used to address questions regarding the lengths, number and success of the different sexual phases within individual trees? For example, a plant that goes through more than one female phase may be able to evaluate pollen donors from the first phase relative to the second phase, and abort fruits developing from lower quality pollen in the first phase in an attempt to hedge bets and obtain better quality pollen in the second phase. Fruit abortion is an established mechanism employed by plants in mate choice[32]. Alternatively, if the Bateman Principle is operating and if maternal fitness is determined only by resource availability while paternal fitness is limited by access to ovules, the male phase should have a more variable distribution in the flowering phenology of an individual, and consequently more variable reproductive success at different times[15,16].

Determination of true male reproductive success is extremely difficult. Thomson and Barrett[15] attempted to overcome this difficulty by assuming that male reproductive success at any given time was a function of the proportion of open male flowers on an individual plant relative to the population. Assumptions about panmixia and sizes of the mating neighbourhood have also to be made. In order to evaluate female reproductive success, experiments are possible in which flowers are pollinated with self- versus cross- pollen, or if self-incompatible, with outcross pollen from single donors versus mixed pollen from multiple donors. The judicious design of such experiments in different female cycles on the same set of individuals may indicate whether there is any effect of such treatments on the occurrence and success of subsequent female phases. One major shortcoming of an experimental approach with a medium-sized tree like *Bridelia* is that we do not know whether localized experimentation on a few branches is sufficient to alter sex expression on these branches. Although Richardson and Stephenson[33] showed that removal of pollen hastened the onset of the female phase in the protandrous *Campanula rapunculoides*, there is only scant knowledge of the feedback systems between and within modules in a plant.

There is much to be understood about sex expression in hermaphroditic plants and about the proximal and ultimate causes for specific strategies[34–36]. Considering the absence of sufficient empirical evidence and the mushrooming of theoretical models, Givnish[7] despaired of the often selective invocation of factors to explain evolutionary scenarios, and thereby the use of arguments by *deus ex machina* instead of rigorous testing of a few models. It is now also recognized that the assumption that hermaphroditic individuals function half as female and half as male is no longer valid[37]. Sexual asymmetry is widespread and is in need of investigation[38]. Goldman and Willson[8] highlight the seeming chaos in the understanding of sex allocation in hermaphroditic plants. They advocate rigorous empirical observation and experimentation since only an augmented data base will provide the large within- and between-species sample sizes necessary to find the patterns and understand the underlying processes. Experimentalists need to find innovative ways of addressing these problems. Systems like those exhibited by *Bridelia retusa* provide opportunities for adding to the general understanding of sex allocation as exhibited in a temporally dioecious species.

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Adaptive evolution and the footprints of history

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The genetic structure of a population at any given time is a reflection of the combined effects of many factors such as past selection history, ongoing selection, ancestry and chance, in the form of random divergence among lineages derived from a common ancestor. In this paper, I describe results from two experiments on the bacterium *Escherichia coli* and the fruit-fly *Drosophila melanogaster*, respectively, in which the contribution of these various factors to adaptive evolution was quantified in rigorous laboratory experiments. In both species, current ongoing selection obliterated the effects of past selection and ancestry on fitness-related traits. In the asexually reproducing *E. coli*, the effects of history on traits not related to fitness were still important after 1000 generations, whereas in the sexually reproducing *D. melanogaster*, traits uncorrelated with fitness showed most variation (~ 95%) to be among individuals within populations. When an effect of history was seen, it was largely due to past selection experienced by a population rather than ancestry *per se*. The time scale of adaptive evolution was much faster in *D. melanogaster*, and the obliteration of the effects of history much more complete, suggesting that genetic recombination may play a major role in removing historical constraints and facilitating adaptive evolution in sexually reproducing species.

BIOLOGICAL systems are characterized by the two interlinked attributes of variation and evolution[1-5]. Although much of the observed variation in populations is undoubtedly environmentally generated, a substantial part of the variation among individuals is due to genetic differences. It is this genetic variation that not only constitutes the raw material for agents of evolutionary change to act upon, but also reflects the outcome of any evolutionary change[4]. In fact, it would not be inaccurate to say that evolution largely consists of the reshaping of patterns of genetic variation within and among populations.

Evolution, it has been said, is what makes biology a different sort of subject from physics, and a key element in evolution is that of historicity[6]. The genetic structure of a population at any given time is a reflection of the combined effects of many factors. The selection pressure faced by a population in the recent past constitutes a directional force that is responsible for the adaptive effects of evolutionary change: natural selection tends to favour the increase in frequency of those genetic variants that make an individual better adapted to the present environment. At the same time, natural selection is constrained by the range of genetic variation available in the population for it to act upon. Since natural selection is essentially little more than the differential reproductive success of different genetic variants in a given environment, it is limited to favouring the increased abundance of the most well-adapted genotype among those extant in the population. The genetic variation available in a population at any time for selection to act upon is itself affected by two major historical factors: the initial genetic composition of the ancestral population, and the past history of selection pressures experienced by the population.

In addition to the directional forces of currently ongoing selection, and the inertial effect of historical constraints upon the range of genetic variation available, any population is also subject to the stochastic influence of random genetic drift, which can cause the genetic composition of the population to change in unpredictable ways. Moreover, selection can itself be constrained by the genetic architecture of fitness traits. Very often different components of fitness exhibit negative genetic correlations among them, thereby preventing natural selection from simultaneously maximizing a number of attributes that would, when considered singly, enhance the fitness of the individual (these are constraints due to pleiotropy in formal population genetic terminology). Add to this genetic constraints on selection due to dominance and epistasis, and it should be clear that the evolutionary trajectory of a population represents the outcome of the resolution of three types of forces: (i) the deterministic force of current natural selection, (ii) the stochastic force of genetic drift, and (iii) the inertial effect due to history, which encompasses effects of ancestry and past selection history[6–10].

History in macroevolution and microevolution

Discussion of the role of history in constraining evolution has typically been restricted to studies of macroevolution (evolution at the species level and above)[11–13]. Developmental or *bauplan* constraints and other forms of phylogenetic inertia have typically been invoked by workers whose primary interest is in speciation, or in the morphological correlates of evolution of taxonomic groups above the level of species[6,7,12,13]. Early microevolutionary studies, in which the focus was on quantifying adaptive evolutionary change within and among populations of a species, tended to neglect the role of history and chance in adaptation and, instead, focused narrowly on the effects of selection (reviewed by Mayr[12]). There is, however, no reason to believe *a priori* that historicity is not important in microevolution. Indeed, the importance of contingent and random factors to adaptive evolution is implicit in Sewall Wright's shifting balance theory[14], first laid out in 1931. The neglect of historicity in microevolutionary studies, thus, has largely been a matter of convenience rather than ignorance. This neglect, nevertheless, has been unfortunate because only in studies at the microevolutionary level do we attain the degree of resolution required to quantitatively assess the role of historical factors in adaptive evolution; macroevolutionary studies, by the very nature of their subject matter, are relatively qualitative and speculative.

In more recent times, the importance of historical factors, such as ancestry, and chance to adaptation at the microevolutionary level has been explicitly considered in the design and interpretation of laboratory experiments on the evolutionary genetics of life-histories[15–17], inter-specific competitors[10,18], and ecological specialization[19]. Similarly, the importance of historicity and chance in determining patterns of within-species diversity in nature has also been receiving increasing attention from ecologists (reviewed by Thompson[20]). It is only in the last two years, however, that there have been any attempts to directly and quantitatively assess the relative contributions of natural selection, history and chance to adaptive evolution. The first such study was carried out by Richard Lenski and his co-workers, who attempted to quantify the role played by adaptation, history and chance in determining genetic variation in populations of the bacterium *Escherichia coli* adapting to novel environments[21]. The only other study of this type, and the first on a eukaryotic, sexually reproducing, and outbreeding species, was conducted by Laurence Mueller and myself[22]. We used populations of fruit-flies (*Drosophila melanogaster*) to try and measure the relative contributions of ongoing selection, past selection history, ancestry and chance to adaptive evolution in populations adapting to various densities.

Selection, chance and history in adaptation in bacteria

In two separate experiments, Lenski and his co-workers studied adaptation to novel nutritional and temperature regimes in populations of *E. coli* over 1000 generations[21]. In one experiment, 12 populations of *E. coli* were derived from a common ancestral population and propagated for 2000 generations in glucose-limited medium[23]. After 200 generations, these populations had similar fitness (assayed through competition versus the common ancestral population) on glucose as the carbohydrate resource, but differed substantially in their fitness in a medium where maltose was the sole carbohydrate. From each of these 12 populations, 3 replicate populations were derived and these 36 experimental populations were then reared on maltose-limited medium for a further 1000 generations. After 1000 generations, the fitness of these 36 populations on maltose-limited medium was again assayed. Cell size on maltose-limited medium was also assayed before and after the

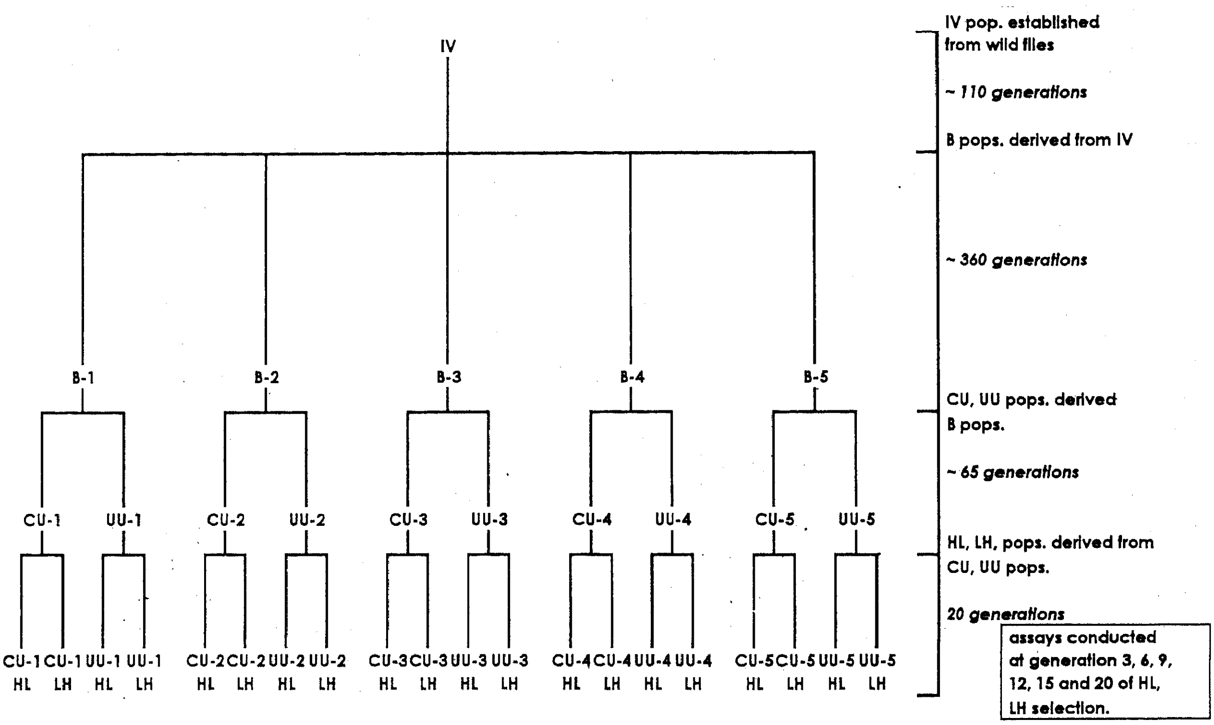

Figure 1. The genealogy of, and past selection pressure experienced by, the 20 populations of *D. melanogaster* used in the study by Joshi, Castillo and Mueller[22]. The IV population, and the five B populations were maintained at low larval density of 60–80 larvae per 8-dram vial.

1000 generations on maltose as carbohydrate source, and was seen to be uncorrelated with fitness. The entire experiment (over 3000 generations) was conducted at 37°C. Using a nested analysis of variance (ANOVA), it was possible to assess, for both fitness and cell size (a trait uncorrelated with fitness), the degree to which the final phenotypes of the 36 populations depended upon (i) history, i.e. differences that arose during the 2000 generations on glucose, (ii) chance, i.e. divergence among the 3 replicates in each of the 12 sets of populations during the 1000 generations on maltose, and (iii) adaptation, i.e. systematic changes in mean phenotype irrespective of initial genotype.

A similar experiment on adaptation to a novel temperature regime was also initiated with one of the 12 populations propagated for 2000 generations on glucose-limited medium at 37°C. From this population, 4 sets of 6 populations each were derived and subjected to different temperature regimes (32°C, 37°C, 42°C, and daily alteration between 32°C and 42°C) for 2000 generations. After these 2000 generations, each of these 24 populations exhibited adaptation to the specific temperature regime they experienced. Derivatives of each of these 24 experimental populations were then maintained at 20°C for 1000 generations. Once again, fitness and cell size at 20°C were assayed before and after the 1000 generations of adaptation to this novel environment.

The results obtained from these two experiments were qualitatively similar. In both cases, at the beginning of the 1000 generations in a novel environment, the contribution of adaptation to fitness and cell size was, by definition, zero. After 1000 generations, the contribution of adaptation to fitness had markedly increased. In the case of adaptation to maltose, the contribution of history and chance to fitness was almost negligible after 1000 generations of evolution in a maltose-limited medium. The contributions of adaptation, history and chance to cell size in maltose-limited medium were of comparable magnitude (in fact, the mean contribution of adaptation, though at par with those of history and chance, did not differ significantly from zero). In the case of adaptation to novel temperature, too, the contribution of adaptation to fitness at 20°C was significantly greater than the combined contribution of history and chance, despite a significant increase over 1000 generations in the latter. In this experiment, the contribution of history was, in part, due to the varying past selection experienced by different populations, with those that had experienced lower temperatures during the preceding 2000 generations being relatively more fit at 20°C. The contribution of history and chance to cell size at 20°C remained essentially unchanged over the 1000 generations of adaptation. Although the contribution of adaptation to cell size increased significantly over 1000 generations at 20°C, it was significantly less than the combined effect of history and chance at the end of the experiment.

The results obtained by Lenski and his co-workers, thus, clearly showed that, at least in clonally propagating populations of *E. coli*, 'the footprint of history may be obliterated for traits that are subject to strong selection, whereas the effect of history is preserved in traits that are less important.'[21] Although this path-breaking experiment was carried out with exemplary rigour, it nevertheless had several shortcomings, partly due to the nature of the experimental organism itself:

(1) Only two observations were made (before and after 1000 generations of adaptation), making it difficult to study the time course of changes in the relative contributions of adaptation, history and chance.
(2) Bacteria are asexual and, hence, depend largely upon mutations to generate the genetic variation that selection can act upon. In sexually reproducing, outbred populations, where recombination plays a major role in generating abundant genetic variation each generation, the rate of evolutionary changes similar to those seen in *E. coli* may be much greater per generation.
(3) It was not possible to clearly differentiate between the respective roles of ancestry and past selection history in determining the contribution ascribed to history.
(4) Bacterial populations being very large, the role of genetic drift in determining the contribution of chance to adaptation in bacteria may be much less than it would be in many eukaryotes, whose populations are typically, orders of magnitude smaller.

Selection, chance and history in adaptation in fruit-flies

Our experiment on *Drosophila* populations[22] avoided the above-mentioned shortcomings, with a slightly more complex design in which relative contributions of different factors to variation in a set of adapting populations were studied at regular intervals, in order to get a better idea of the time course of changes in these contributions. We used a set of 20 laboratory populations of *D. melanogaster*, whose ancestry and past-selection history, in the specific context of density, was known for over 550 generations (Figure 1). The 20 experimental populations were set up by deriving two populations from each of a set of 10 populations that had been subjected to either low (~ 70 larvae per 8-dram vial: five UU populations) or high (> 1000 larvae per 6-dram vial: five CU populations) larval density for the preceding 65 generations. Each pair of CU and UU populations (e.g. CU-1, UU-1) had, in turn, been derived from one of the five B-populations of Rose[24], which had been in the laboratory for ~ 360 generations following their derivation from a common ancestor, the IV population[24]. The maintenance regimes and evolutionary differentiation of the UU and CU populations that served as the starting point of our experiment have previously been described in detail[17,25,26], so I will restrict my discussion here to only those details as are pertinent to the experiment being described.

In the course of 65 generations of evolution under crowded larval conditions, the CU populations had evolved a higher larval feeding rate, as compared to their low density controls, the UU populations[17]. Larval feeding rate in *Drosophila* is very strongly correlated with competitive ability and, therefore, fitness, under crowded larval conditions[27]. Pupation height, the height above the food surface that the larvae pupate, had not significantly changed in the CU populations[17], suggesting that it was not significantly related to fitness at high larval density in these populations, although this trait had earlier been seen to evolve in crowded populations of *Drosophila*[28–30].

In our experiment, two populations were derived from each CU and UU population, and subjected to either crowded (LH populations) or uncrowded (HL populations) larval conditions for 20 generations. The experiment thus involved populations designated CU-HL, CU-LH, UU-HL and UU-LH, representing various combinations larval density experienced during past and ongoing selection (e.g. CU-HL populations had undergone larval crowding in the past, but during the course of the experiment were subjected to reverse selection by being kept at low density). Both larval feeding rate (20 larvae per population) and pupation height (5 vials of ~50 lar-

Table 1. Interpretation of different variance components in terms of the contribution of ancestry, chance, past selection and ongoing selection to adaptation to larval density level in 20 populations of *D. melanogaster*

Effect in variance components model	Interpretation
Block (numerical subscript)	Initial genetic composition, or ancestry, as a result of divergence among the five B-populations over ~ 360 generations.
Selection (CU *versus* UU)	Past selection for 65 generations
Treatment (HL *versus* LH)	Current selection during the 20 generations of the experiment.
Block × Selection	Random divergence among lineages during 65 generations of CU, UU selection
Block × Selection × Treatment	Random divergence among lineages during 20 generations of HL, LH selection

Adapted from ref. 22.

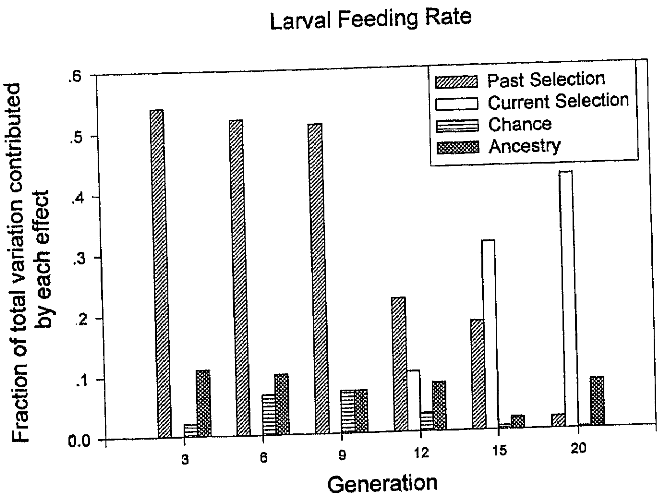

Figure 2. The relative contribution of past and current (ongoing) selection, chance (random divergence among lineages) and ancestry (initial genetic composition) to variation in larval feeding rate in 20 populations of *D. melanogaster* undergoing adaptation to different larval densities (data from ref. 22). Many interaction effects, and the error terms, are not depicted in the interests of clarity.

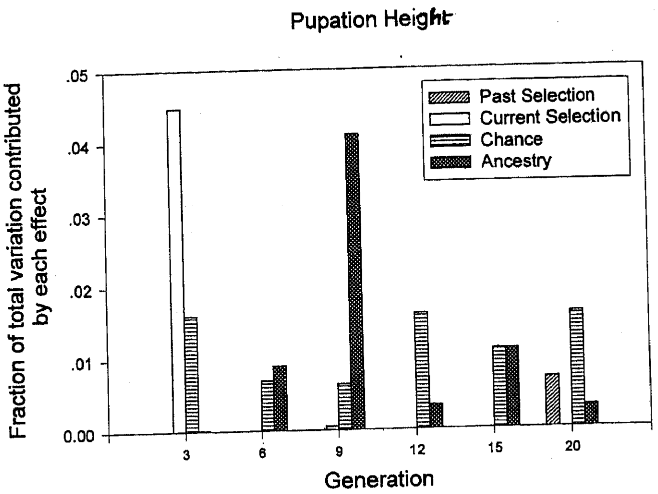

Figure 3. The relative contribution of past and current (ongoing) selection, chance (random divergence among lineages) and ancestry (initial genetic composition) to variation in pupation height in 20 populations of *D. melanogaster* undergoing adaptation to different larval densities (data from ref. 22). Many interaction effects, and the error terms, are not depicted in the interest of clarity.

vae per population) were assayed at generations 0, 3, 6, 9, 12, 15 and 20 of the experiment. The data for each generation were subjected to a variance components analysis from which an estimate could be made of the contribution of various factors to phenotypic variation in these populations at various stages of the experiment (Table 1). Due to the pattern of relatedness among the CU and UU populations (CU_i and UU_i are more closely related to each other than either of them is to other populations with which they share the same selection regime, $i = 1..5$), sets of CU-HL, CU-LH, UU-HL and UU-LH populations, matched by subscripted indices, were treated as blocks in the analysis.

The results for larval feeding rate from this experiment clearly show that by about 20 generations, the primary contribution to variation in feeding rate in the set of 20 populations was due to current or ongoing selection (Figure 2). During the first 10 generations, past selection history (CU or UU) played a major role in determining the feeding rate of a population. The effects of chance divergence over 65 generations, as well as over the course of the experiment, were negligible; the effect of ancestry remained more or less constant over the 20 generations of the experiment (Figure 2). Thus, adaptation to present conditions largely determined the larval feeding rate of the populations after 20 generations, regardless of their prior history of high or low larval density. In the case of pupation height, the major contribution to variation at all generations was actually that of the error term, representing variation among individuals within populations (data not shown). At no generation in which observations were made did the combined contribution of past and current selection, ancestry and chance exceed 5% of the total variation in the set of 20 populations (Figure 3). Moreover, no particular pattern in the relative contribution of these various factors to variation in pupation height could be discerned (Figure 3). The role of history, in the form of ancestry and past selection, and random divergence among lineages, would, therefore, appear to be of far less importance to adaptive evolution in *Drosophila* populations, as compared to the asexually reproducing *E. coli*.

Conclusion: how deep are the footprints of history and what causes them ?

There is some similarity in the results from *E. coli*[21] and *D. melanogaster*[22], most notably the fact that for fitness (or a trait strongly correlated with fitness), the effect of current ongoing selection tends to obliterate the effects of historical and random effects such as ancestry, past selection and random divergence among lineages. This is, of course, not terribly surprising, considering the well known role of natural selection in adaptive evolution. What is more interesting is to look at some of the differences in the results from *E. coli* and *D. melanogaster*, in order to get some appreciation of how the pattern and tempo of evolution may vary in asexually and sexually reproducing species.

In the case of *E. coli*, history played a major role in determining variation for cell size, a trait uncorrelated with fitness, even after 1000 generations. The footprint of history was, thus, deep enough to withstand 1000 generations of random divergence among lineages, in the absence of any strong selection. This may be partly due to the fact that the effective population size of bacterial cultures is extremely large, thus minimizing the role of random genetic drift. Moreover, the asexual bacteria rely on new mutations, and possibly transposition events, to generate new genetic variation, thus slowing down considerably the rate at which genetic variation arises (contrast this to a sexually reproducing species where, even in the absence of mutation, recombination will generate numerous new genotypes each generation).

In the sexually reproducing *D. melanogaster* populations, the footprints of history turned out to be rather shallow, even for pupation height, a trait uncorrelated with fitness under the conditions of the experiment. Indeed for this non-adaptive trait, the major contribution turned out to be that of variation among individuals within populations: eloquent testimony to the power of recombination to obliterate the imprint of past events from genomes. Even in the case of larval feeding rate, which is highly correlated with fitness, the time scale over which the footprints of history were wiped out was far more rapid than in *E. coli*: in 20 generations the contribution of history to variation in the system declined from about 65% to about 12%. Moreover, the effect of history, while it lasted, was primarily a consequence of past selection experienced by the populations rather than ancestry, as in the case of temperature adaptation in *E. coli*. Thus, the footprints of history, at least in sexually reproducing species, would, metaphorically speaking, appear to be no more than transient impressions on genomic dust. With a change in selection pressure, the genetic structure of a population is rapidly shuffled and reorganized, much as what happens to a particular sequence of sand dunes upon a sudden change in the direction of the wind.

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Social evolution – has nature ever rewound the tape?

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Social insects such as ants, bees, wasps and termites exhibit extreme forms of altruism where some individuals remain sterile and assist other individuals in reproduction. Hamilton's inclusive fitness theory provides a powerful framework for investigating the evolution of such altruism. Using the paper wasp *Ropalidia marginata*, we have quantified and delineated the role of ecological, physiological, genetic and demographic factors in social evolution. An interesting feature of the models we have developed is their symmetry so that either altruism or selfishness can evolve, depending on the numerical values of various parameters. This suggests that selfish/solitary behaviour must occasionally re-emerge even from the eusocial state. It is useful to contemplate expected intermediate states during such potential reversals. We can perhaps envisage three successive steps in such a hypothetical process: i) workers revolt against the hegemony of the queen and challenge her status as the sole reproductive, ii) workers stop producing queens and one or more of them function as egg layers (functional queen/s) capable of producing both haploid as well as diploid offspring and iii) social evolution reverses completely so that a eusocial species becomes solitary, at least facultatively. It appears that the third step, namely transition from eusociality to the solitary state, is rare and has been restricted to transitions from the primitively eusocial state only. The absence of transitions from the highly eusocial state to the solitary state may be attributed to a number of 'preventing mechanisms' such as (a) queen control of workers (b) loss of spermathecae and ability to mate (c) morphological specialization (d) caste polyethism and (e) homeostasis, which must each make the transition difficult and, taken together, perhaps very difficult. However, the discovery of a transition from the highly eusocial to the solitary state can hardly be ruled out, given that little or no effort has gone into its detection. In this paper I discuss social evolution and its possible reversal and cite potential examples of stages in the transition from the social to the solitary.

Altruism and eusociality

Competitive selfishness is the corner-stone of natural selection. Nevertheless, we see many instances of the evolution of cooperation and even altruism in the animal kingdom. In studies of animal behaviour, altruism is defined as any act which increases the fitness of the recipient of the altruistic act and decreases the fitness of the actor. While there are some examples of altruism in birds and mammals, especially in cooperative breeding species, the most extreme forms of altruism are to be found among eusocial insects such as ants, bees, wasps and termites[1]. Eusociality, considered as the most developed form of social life, is characterized by (1) cooperative brood care, (2) differentiation of colony members into fertile reproductive castes and sterile worker castes and (3) the coexistence within a nest of individuals belonging to more than one generation[2,3]. Among the eusocial insects, some are considered even more advanced than the rest. In these so-called highly eusocial species, queens and workers are morphologically differentiated and caste determination, which takes place before the adult stage, is essentially irreversible. The less advanced, so-called primitively eusocial species, have the opposite set of characters – little or no morphological caste differentiation coupled with flexibility and reversibility of caste roles[3]. Although there may not be a very sharp dividing line between the primitively and the highly eusocial groups, termites, ants, honeybees, vespine wasps and most polybiine wasps are considered to be highly eusocial[3]. Conversely stenogastrine wasps, independent founding polistine wasps, halictine bees and meliponine bees, as well as the recently discovered eusocial aphids[4–6], thrips[7], ambrosia beetles[8], shrimps[9] and the naked mole rat[10,11] are all considered to be primitively eusocial.

The evolution of altruism and of eusociality have been the subject of extensive theoretical and empirical investigations during the last three decades[1,12–18]. In contrast, the possibility of reversal of social evolution, namely, the transition from the eusocial to the solitary has received surprisingly little attention. During a brief discussion, Wilson[3] concludes that although the solitary habit may have sometimes evolved from primitively eusocial ancestors, highly eusocial species have probably reached a point of no return. In halictine bees, however, which have a great diversity of social organizations, frequent alternations occur between solitary and eusocial states, both between different habitats and between different species within a genus. Halictine bee researchers have therefore been long concerned about transition from the primitively eusocial to the solitary state[19–24]. My own interest in such reverse social evolution, which I metaphorically call 'rewinding the tape' arises from our investigations of traditional 'forward' social evolution. To set the stage, therefore, I will first briefly describe our attempts and some modest success in understanding the evolutionary forces that promote the evolution of eusociality.

Hamilton's inclusive fitness theory

As is now well known, Hamilton[25,26] showed that altruism will not necessarily be eliminated by natural selection if it is directed towards close genetic relatives. If fitness is defined not simply as the number of offspring, but as the number of copies of one's genes that are transmitted to the next generation, aiding genetic relatives who also carry copies of one's genes, is an alternative, equally legitimate way of enhancing one's fitness. Hamilton therefore proposed that we should be concerned with inclusive fitness which combines propagation of genes through production of one's own offspring and through aiding genetic relatives. More formally, Hamilton showed that an altruist allele would be favoured by natural selection if:

$$b/c > 1/r, \tag{1}$$

where b is the benefit to the recipient of the altruistic act, c is the cost to the altruist and r is the coefficient of genetic relatedness between the altruist and the recipient. This so-called Hamilton's rule has proved to be a powerful theoretical framework for investigating the evolution of altruism.

Ropalidia marginata

Ropalidia marginata is a polistine wasp abundantly distributed in peninsular India. New colonies may be initiated throughout the year by one or a group of female wasps. In single foundress colonies, the lone female builds a nest, lays eggs, cares for her larvae by foraging for them and guarding the nest and thus brings at least the first batch of her offspring to adulthood, unaided by conspecifics, much like a solitary wasp. In multiple foundress colonies, however, there is a dominance hierarchy, leading to division of labour such that only one individual functions as the queen and lays all eggs while the rest function as workers and perform all the tasks involved in colony maintenance and brood care. Daughters eclosing from single and multiple foundress nests may either leave to start their own new colonies or may stay back and function as workers in their natal nests. This means that wasps have the option of reproducing (by becoming solitary nest foundresses) or of becoming workers (by staying back in their natal nest or by joining newly founded nests as subordinates). *Ropalidia marginata* is therefore ideally suited for empirical measurements of the costs and benefits of solitary and social life and to the use of Hamilton's inclusive fitness theory to gain some insight into the evolution of altruism[27]. The task before us, therefore, has been to measure and contrast the inclusive fitness of a solitary nest founding wasp on the one hand and a wasp choosing to function as a worker in her mother's or sister's nest, on the other hand[18].

Factors promoting the evolution of altruism

For our purpose, Hamilton's rule can be rewritten as:

$$\beta \rho \sigma > b r s, \tag{2}$$

where β is the intrinsic productivity of a worker, defined as the number of individuals she can rear to adulthood provided she survives for their entire developmental period, ρ is the coefficient of genetic relatedness of a worker to the brood she rears and σ is the demographic correction factor for a worker, defined as that factor by which a worker's intrinsic productivity should be devalued because of the probability of her dying before the brood under her care completes development. The corresponding parameters for a solitary foundress are b, r and s (refs 28–30). Clearly at least 3 classes of factors, exemplified by β, ρ and σ or b, r and s can contribute to inequality (2): ecological or physiological factors ($\beta > b$); genetic factors ($\rho > r$) and demographic factors ($\sigma > s$)[18,30].

During the last ten years or so, we have been engaged in attempts to assess the relative roles of ecological, physiological, genetic and demographic factors in the evolution of worker behaviour in *Ropalidia marginata*. This work is continuing and has been reviewed from time to time[15,16,18,27,31,32]. In summary, we have concluded that worker behaviour in *R. marginata* is favoured because the ecological, physiological and demographic factors tend to tilt the balance in favour of worker behaviour although the genetic relatedness terms, if anything, favour solitary nesting behaviour.

We are currently in the process of considering all of these factors simultaneously and of developing a unified model for the evolution of the observed mix of worker behaviour and solitary founding in *R. marginata*. As seen from inequality (2), our model has six parameters, namely β, ρ, σ, b, r, and s. To put it very briefly, we have estimated the values of these parameters as, $\beta = 12.3$, $\rho = 0.11$ to 0.23, $\sigma = 0.43$, $b = 4.2$ to 12.3, $r = 0.5$ (this is assumed) and $s = 0.01$ to 0.12 (refs 18, 29, 30, 33–35). Exploring the entire parameter space over the range of these numerical values, we find that the observed parameter space includes regions where the net balance is in favour of worker behaviour and also where the net balance is in favour of solitary nesting behaviour. This is satisfying because both strategies are known to coexist in populations of *R. marginata*[36]. An overwhelming proportion of the parameter space favours the worker strategy and this is even more satisfying because solitary nests are notoriously hard to find. To be more precise, worker behaviour is favoured in 96.2% of the parameter space while the solitary nesting behaviour is only favoured in 3.8% of the parameter space[37]. In an extensive empirical investigation of colony founding in *R. marginata* we recently estimated that 92.5 to 94% of the wasps in *R. marginata* populations naturally choose to nest in groups while only 4.6 to 5.7% choose the solitary nesting strategy[36]. This extremely close fit between the observed and expected proportions for the solitary and worker strategies is most impressive. However, that our model has certainly not considered several other factors which must also play a role, is a very sobering thought!

Reverse social evolution or, the evolution of selfishness

What can the foregoing analysis of the evolutionary forces favouring worker behaviour tell us about the possibility of reversal of social evolution? As mentioned in the beginning, there has seldom been an attempt to examine if selfishness can re-emerge in a group of altruists. In our context this is equivalent to asking whether social insect species can retrace their evolutionary pathway and eventually become solitary. It is obvious that Hamilton's rule itself provides a theoretical framework to explore the emergence of selfishness. It follows, therefore, that inequality (2) can again become a useful tool if we reverse the inequality sign. In other words, we could say that selfishness would be favoured over altruism if:

$$\beta \rho \sigma < b r s. \qquad (3)$$

Indeed, this is exactly why solitary nesting is favoured over worker behaviour in *R. marginata* in a small portion of the parameter space that we have explored above. Thus the inclusive fitness model developed here for understanding the evolution of altruism is symmetrical with reference to direction and may favour the evolution of altruism or selfishness depending merely on the numerical values of the parameters. It is this symmetry in the model that has created in me an interest in the possibility of reverse social evolution. That solitary nesting strategy is favoured in some portion of the parameter space, in the case of *R. marginata,* is itself, however, not sufficient evidence of reverse social evolution. To demonstrate this, we must find the solitary nesting strategy in the descendants of an obligatorily eusocial ancestor or the loss of eusociality in the descendants of an ancestor that was at least facultatively eusocial.

Can solitary behaviour re-emerge in highly eusocial species?

In spite of admitting that 'social evolution can be reversed and a eusocial species can revert to a solitary condition', Wilson[3] concludes that 'there are reasons for believing that a point of no return (in social evolution) does indeed exist'. The reason for this dichotomy is that all suspected examples of possible reversion from eusociality to the solitary state concern primitively eusocial species and as Wilson[3] says, 'the highest insect societies have lost elements of behaviour that would be very difficult to reattain in evolution'. Is it pointless then to persist with the question of the possibility of reversal of the highly eusocial state? I believe that there is still some merit in persisting with the question and suggest a three-pronged attack: (i) intensify investigations of suspected reversals from the primitively eusocial state, (ii) identify and investigate possible intermediate states in potential reversals from the highly eusocial state and (iii) identify and investigate 'preventing mechanisms' or factors responsible for the rarity of reverse social evolution both at the primitively eusocial as well as the highly eusocial level. Let us proceed in the reverse order!

Preventing mechanisms

While discussing the evolution of eusociality, it is customary to talk about additional enabling mechanisms over and above the parameters in inequality (2). In the context of eusociality in the Hymenoptera, the sting, the nest and advanced parental care are the three most commonly discussed enabling mechanisms[13,14]. In other words, the presence of the sting which greatly facilitates defence of large aggregations of adults and helpless larvae, the habit of building elaborate nests that protect and provide environmental control and the possession of well-developed abilities of extended parental care are all expected to make it easier for sociality to evolve if inequality (2) is satisfied. It is reasonable, therefore, that we must consider corresponding 'preventing mechanisms' that might make it difficult for solitary behaviour to re-emerge even if inequality (3) is satisfied. Based on the discussion of this question in Wilson[3] and what we know about social insects today, there appear to be at least five major preventing mechanisms that make it difficult or impossible for solitary behaviour to re-emerge from the highly eusocial state. Of course, all five factors are not likely to be equally powerful –

morphology would be hardest to reverse and behaviour would be the easiest with chemistry somewhere in between.

Queen control of workers

A fundamental property of all social insects colonies is the control of worker behaviour and especially worker reproduction by the queens[3,17,38]. This is accomplished by queen pheromones in the advanced social insects whereas in the primitively eusocial species it may be accomplished entirely by direct physical interactions. In the case of the honey bee, for example, the queen produces a host of chemical substances that influence the behaviour and physiology of the workers in her colony. Owing to the fact that each colony consists of a single queen and many thousand workers, communication between the queen and her workers is, as expected, primarily mediated by chemicals. The well-known effects of queen pheromones on workers include rapid detection of the presence or absence of the queen. A retinue of some 8 to 10 workers, the composition of which changes every few minutes, feeds and licks the queen and thereby acquires the queen pheromones and passes them on to other workers. The pheromones also inhibit the development of worker ovaries and stimulate building and foraging activities. Workers of a queen-right colony almost never lay eggs. Instead, they engage in building combs, feeding the larvae, grooming and feeding the queen, protecting the hive from intruders, foraging, and storing honey and pollen[39]. Similar chemical control of workers by the queens probably occurs in all highly eusocial species although the nature of the chemicals and the extent of their effects remain to be studied in most cases. The alternative point of view that queen pheromones in highly eusocial insects are not agents of control but are signals that workers use to forgo reproduction which is best left to the queens[40,41] does not alter our argument that queen pheromones can act as a preventing mechanism for reverse social evolution. Situations where it is to the advantage of the workers to remain sterile and let the queens lay eggs are hardly expected to be conducive for workers to revolt against the hegemony of the queen/s.

In the primitively eusocial wasp *Polistes fuscatus* on the other hand, the queen is behaviourally extremely active and thus regulates the behaviour of workers. The fact that worker activity is stimulated by queen activity was most strikingly demonstrated in an experiment whereby introduction of a cooled inactive queen into a colony, depressed worker activity even more than the mere absence of the queen[42]. Queen control of workers must surely serve to prevent reversal of social evolution and the strength of such prevention must depend on the strength of queen control. It is reasonable to think that workers in primitively eusocial species can relatively more easily escape from the nudging and biting of the queen compared to a honey bee worker whose behaviour and physiology are strongly influenced by the presence of queen pheromones.

Loss of spermatheca and ability to mate

In most highly eusocial species workers cannot mate and store sperm. In the queens, the spermatheca, a small sac-like diverticulum which opens into the vagina dorsally, acts as a reservoir for sperm and has associated glands to secrete nutrients and keep the sperm viable. In most highly eusocial species, workers have reduced and non-functional spermathecae. Barring one or two exceptions (see below) this completely prevents workers from producing female offspring. In many species workers retain rudimentary ovaries and can lay a few haploid eggs upon the death of the queen[43]. However this does not give them complete freedom from the queen and this has probably been a major preventing factor for the reversal of social evolution.

Morphological specialization

With very few exceptions, highly eusocial insects, especially ants and termites, are unparalleled in the extent of intra-species and intra-sexual variations in size and allometry. The most widespread differentiation is between fertile reproductives (queens) and sterile workers[3]. In the ants and termites, sub-groups among workers may also be morphologically differentiated into major (soldier), minor and media workers[44–46]. Such morphological caste differentiation can be so extreme that different castes, if encountered separately, may get classified as different species[47]. The greatest size variation has been recorded in the Asian marauder ant *Pheidologeton diversus*[48] where major workers weigh 500 times as much as minor workers and have a head width 10 times as large. Such morphological specialization of workers for non-reproductive and non-colony founding roles has also probably been a significant factor in making reverse social evolution difficult.

Caste polyethism

Division of labour is a striking feature of colony organization in most social insects. While the most fundamental division of labour is between the reproductive and the worker caste, further division of labour within the workers is accomplished either by physical or by temporal means or by combination of both[3,45]. When castes are physically differentiated, behaviour is correlated with size and when castes are temporally differentiated, behaviour is correlated with age. Physical castes are most strongly developed in termites[49] and ants[46], although temporal castes are also seen. Temporal castes are most strongly developed in stingless bees[50] and honey bees[51]. In halictine bees[52], bumblebees[53] and wasps[54], the correlation of behaviour is less pronounced both with size and with age. There is now evidence that age polyethism can precede the evolution of morphological differentiation between queens and workers[55]. Although such caste polyethism is relatively flexible and allows readjustment of behaviour in response to unusual age or size distribution of workers and unusual levels of demand for work, behavioural specialization based on size and/or age is expected to put some restrictions on the ability of workers to revert to a solitary mode of existence.

Homeostasis

The ability of social insects to regulate the environment of their nest, sometimes called social homeostasis, is most impressive, especially in the highly eusocial species[3]. While such homeostasis has permitted social insects to gain a considerable measure of independence from the environment, it has also made them dependent on the nearly constant conditions prevailing in their nests. It is a common observation that individual bees and ants that lose their way to the nest die very quickly. This dependence on the homeostatic conditions provided by the nest is also likely to have been a preventing factor in the reversal of social evolution.

Intermediate steps in reverse social evolution

As a consequence of the unlikelihood of complete reversal from the highly eusocial state to the solitary state, it is useful to contemplate possible intermediate states during potential reversals. We can perhaps envisage three successive steps in such a hypothetical process. 1) Workers revolt against the hegemony of the queen (whether morphologically differentiated queens or merely functional queens) and challenge her status as the only reproductive individual, 2) workers stop producing queens and one or more of them function as egg layers capable of producing both haploid as well as diploid offspring (functional queens) and 3) social evolution reverses completely so that the species becomes solitary.

Thelytoky

There are at least two good examples of workers having successfully revolted against the hegemony of the queen and having challenged her status as the only reproductive individual. This appears to have been accomplished by a phenomenon termed thelytokous parthenogenesis which permits unmated workers to lay diploid, viable eggs which will develop into fertile adult females and, given the right conditions, will develop into morphologically differentiated queens. This has happened in the Cape honey bee, *Apis mellifera capensis*, a unique race of honey bees found at the tip of South Africa. Unlike in any other race of honey bees, virgin workers in the Cape honey bee lay diploid eggs by thelytokous parthenogenesis and the laying workers have many queen-like characteristics including well-developed ovaries with many ovarioles per ovary, well-developed spermathecae, production of queen-like pheromones, possession of queen-like retinue of workers, high reproductive dominance and ability to inhibit production of queen cells[56–60]. Another example is the ant *Cataglyphis cursor*[61–64] where, like in the cape honey bee, thelytoky is facultative and has not led to the elimination of the queen.

Queenless ants

There are several examples among ants where, on an evolutionary time scale, workers have stopped producing morphologically differentiated queens so that present-day colonies consist of one or more egg-laying workers who can produce both haploid and diploid offspring (functional queens). In many ponerine ants, workers routinely mate and reproduce and there has sometimes been a permanent loss of true queens. In some species a single, mated, laying worker, called the gamergate, suppresses ovarian development of all nestmates and functionally behaves like a queen[65–68]. The ponerine workers appear to have accomplished this unusual evolutionary feat on account of their morphological primitiveness which includes retention of a functional spermatheca. Nevertheless, queenlessness (and perhaps also the presence of mated workers) appears, from phylogenetic studies[69] to be a derived condition (Figure 1), justifying this as an example of an intermediate stage in reverse social evolution. Despite the loss of spermatheca, in the Japanese myrmicine ant *Pristomyrmex pungens*, the same result appears to have been accomplished by the evolution of thelytoky as in the Cape honey bee and the ant *Cataglyphis cursor*. However because thelytokous parthenogenesis is obligatory in *Pristomyrmex pungens*, I place this species in the second step of reverse social evolution, ahead of the Cape honey bee and of *Cataglyphis cursor*. Queens are entirely absent in *P. pungens* and workers are unusual in that each one of them performs egg laying as well as other non-reproductive tasks through a curious mode of division of labour – young workers remain inside the nests and reproduce while older workers come out of the nest and forage[70–72].

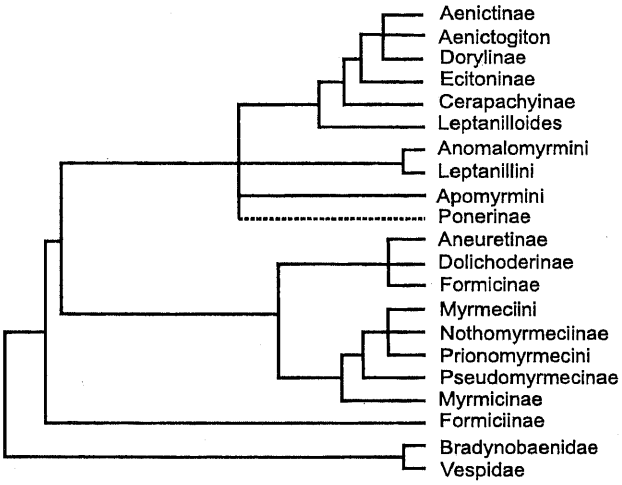

Figure 1. The internal phylogeny of ants as revealed by the strict consensus tree calculated from 72 equally most parsimonious trees resulting from a cladistic analysis of 17 ant taxa and 2 vespoid outgroups. Notice that the position of the morphologically primitive, queenless subfamily Ponerinae indicates that queenlessness in this subfamily appears to be a derived character. (Reproduced with permission from Baroni Urbani and *Systematic Entomology*.)

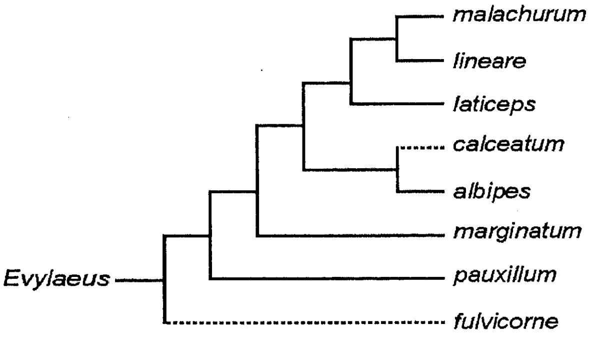

Figure 2. Behavioural evolutionary changes in the halictine sub-genus *Evylaeus* mapped upon the phylogeny derived from allozymes. Notice that solitary behaviour observed in the species *calceatum* is a derived condition. The solitary state in the species *fulvicorne* may also be a derived condition because the sub-genus *Evylaeus* may itself be descended from a eusocial ancestor. (Reproduced with permission from Lawrence Packer and *Behavioural Ecology and Sociobiology*.)

From eusocial to solitary?

As mentioned earlier, the final step of going from a morphologically well-differentiated highly eusocial state, all the way to the solitary state, does not appear to have been taken by any social insect. However, transitions from the primitively eusocial to the solitary are not uncommon in the halictine bees[73]. In the context of the evolution of the Halictinae, Michener[21] suspects that 'eusocial behaviour has arisen repeatedly, dozens or hundreds of times, and that reversion to solitary behaviour is easy'. A particularly clear example emerges from a phylogenetic study of social behaviour and nest architecture in the sweat bee sub-genus *Evylaeus*[74]. When behavioural changes are mapped on to the phylogeny derived from allozymes, it becomes obvious that the solitary state of the species *calceatum* is a condition that is derived from the ancestral eusocial state (Figure 2).

It must be mentioned that in all this discussion, I have not considered a different kind of reversion from the eusocial state namely, reversion to a socially parasitic state, rather than the solitary state. This phenomenon has received considerable attention[75–77] although its integration with the phenomenon of reversion to the solitary state remains to be achieved.

Concluding remarks

Although highly eusocial species may not have reverted entirely to the solitary state, investigation of suspected intermediate states in the process of reversion may lead to an understanding of why reverse social evolution is not so common and might perhaps even lead to the discovery of true reverse social evolution. At the very least, it will lead to a better understanding of why reversal from the highly eusocial state is difficult or impossible.

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A novel observation in the measurement of ultrafast relaxation times using incoherent light

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We present here our experimental results on the measurement of population relaxation times of the excited states of some organic dyes such as erythrosinB, commercial ink and metalloporphyrin using degenerate four wave mixing with incoherent light (DFWM-IL). When the probe beam in the phase conjugate configuration is delayed, we observe a second peak apart from the coherence peak due to forward pump and the probe beams. The second peak, due to backward pump and the probe beams, is observed only for samples having excited state absorption and has different widths for different samples. From the observed line widths, we estimate the relaxation times of the excited states S_n and S_1.

MANY organic materials, with π electron delocalization, are currently being studied for possible device applications in optical communication and optical data processing[1,2]. Most of these materials due to their complex structure, have a large number of vibrational and rotational bands, leading to very fast nonradiative transitions within each electronic band. These relaxations normally occur in ps and fs domain. Different spectroscopic methods have been developed for measuring these ultrafast relaxation times without the use of fs laser[3]. A technique was proposed by Morita and Yajima[4] which uses incoherent light as the source. This technique has been applied to studies of both dephasing times in fs domain and longitudinal relaxation times in the ps domain[5]. Kobayashi *et al.*[6] derived a generalized formula for calculating the relaxation times when one of the beams is delayed with respect to the other beams. Okamoto[7] extended the work of Morita and Yajima for systems with more than two levels, like organic dyes, having different excited and ground state relaxation times and showed that the population relaxation times can be obtained using non-zero delay of beam 2 also. In this communication, we present our new observations in the measurement of population relaxation times of few organic dyes such as erythrosinB, commercial black ink and porphyrins. A counter propagating geometry for the degenerate four wave mixing using incoherent light (DFWM-IL) has been utilized for these studies. For the first time we observe different line widths as compared to Okamoto's results and these are explained in terms of the relaxation times of different excited states.

A schematic of the experimental setup is shown in Figure 1. Home-built dye laser[8] consists of an oscillator and a single stage amplifier. The laser is pumped by second harmonic of Nd:YAG (Continuum, USA, 6 ns, 10 Hz) with an output of ~ 300 mJ in 1064 nm and ~100 mJ in 532 nm. Using a glass plate, 8% of power is reflected and is used for pumping the oscillator. The remaining power is used for amplification of the signal. The oscillator has a plane mirror and a glass plate (~8% R) combination which produces broad band

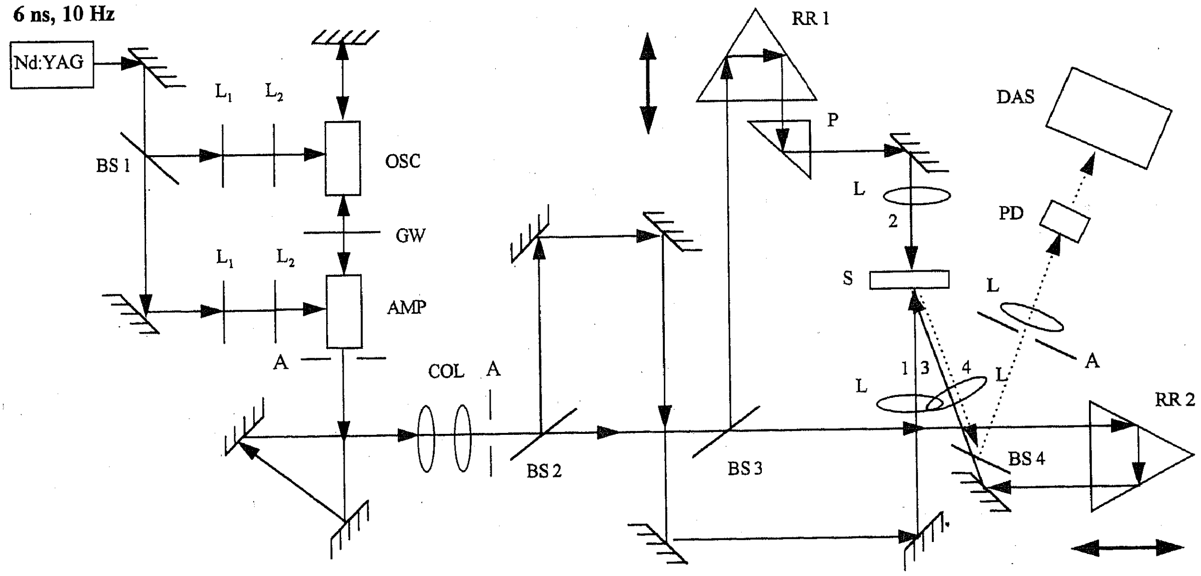

Figure 1. Schematic of the experimental setup. OSC, Oscillator; AMP, Amplifier; RR, Retro reflector; DAS, Data Acquisition System; L_1, Diverging lens; L_2, Cylindrical lens; PD, Photo diode; BS3, BS4, 50-50 Beam splitters; GW, Glass wedge (4% R); Col, Collimater; P, Right angled prism; BS2, 30-70 Beam splitter; L, Converging lens; S, Sample; A, Aperture; BS1, 10-90 Beam splitter.

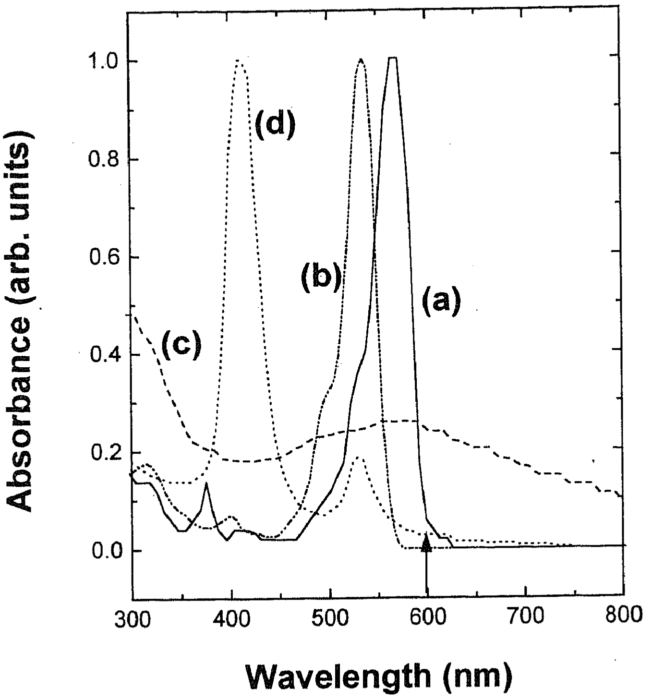

Figure 2. Absorption spectrum of the samples. *a*, RhB; *b*, erythrosinB; *c*, black ink; *d*, CoTTP.

light. FWHM of the output observed is ~ 8 nm (at 595 nm) and the corresponding coherence time (τ_c) calculated is ~ 160 fs. The beam is initially collimated before splitting it into three beams. Two beam splitters (30–70, 50–50) are used to obtain three beams. All the three beams have a diameter of ~ 3 mm. Beam 1 is forward pump ($\mathbf{k}_1$), beam 2 ($\mathbf{k}_2$) is backward pump and beam 3 ($\mathbf{k}_3$) is the probe. Beam 1 is fixed whereas beams 2 and 3 pass through variable delays. Beam 3 makes an angle of ~10° with the forward pump. All the beams are focused into the sample contained in 1 mm/100 μm thick quartz cell using lenses of focal length ~ 20 cm. Beam waist at the focus was found to be ~ 80 μm. The phase conjugate beam (in $-\mathbf{k}_3$ direction) is isolated using a beam splitter and is focused on to a fast photo diode, the output of which is given to the data acquisition system.

The signal is recorded by delaying the beam 3 for different, fixed delays of beam 2 with respect to beam 1. For transient gratings, signal in the direction $\mathbf{k}_s = \mathbf{k}_1 + \mathbf{k}_2 - \mathbf{k}_3$ has contribution from two different gratings[9].

1) $\mathbf{k}_2$ at time t_3 gets diffracted by the grating formed with the fields in directions of $\mathbf{k}_3$ and $\mathbf{k}_1$ at t_1 and t_2 respectively.

2) $\mathbf{k}_1$ at time t_3 gets diffracted by the grating formed with the fields in directions $\mathbf{k}_3$ and $\mathbf{k}_2$ at t_1 and t_2 respectively.

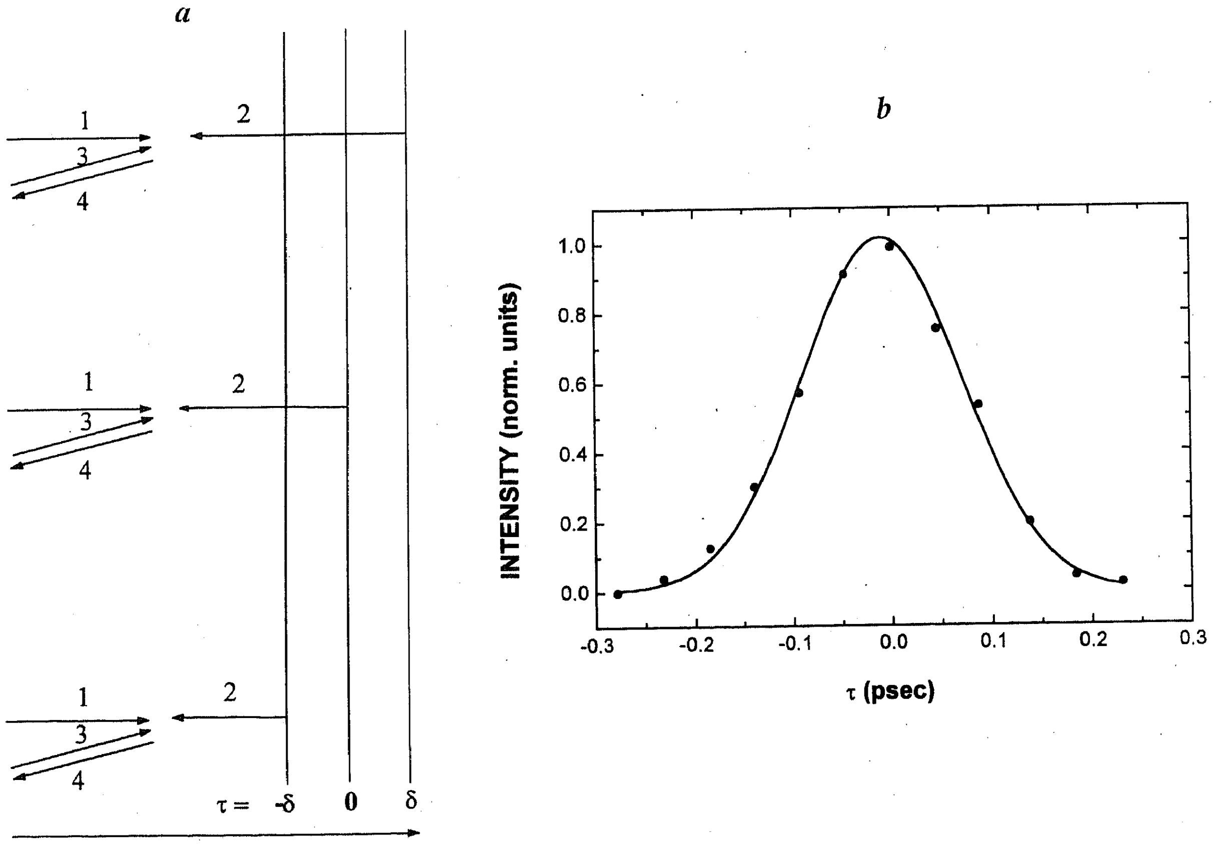

Figure 3. *a*, Pictorial representation of various delays. *b*, Autocorrelation function (RhB in methanol).

The absorption spectra of all the samples are shown in Figure 2. The delay time between $\mathbf{k}_1$ and $\mathbf{k}_2$ is denoted as δ and that of $\mathbf{k}_1$ and $\mathbf{k}_3$ as τ (Figure 3 *a*). Figure 3 *b* depicts the autocorrelation curve of RhB in methanol and the correlation time was found to be ~160 fs. Figure 4 shows time resolved profiles, for different values of δ, in RhB (in methanol), erythrosinB (in menthanol), black ink (in distilled water) and cobalt tetra tolyl porphyrin (CoTTP in chloroform). All the profiles have two peaks except the one in RhB. Peak 1 is due to the coherence of beams 1 & 3 and has a width of ~170–180 fs. The second peak is due to the coherence of beams 2 & 3 and has different widths. The widths (FWHM) of the second peak are ~ 180 fs in erythrosinB, ~ 1.5 ps in black ink, ~ 3 ps in porphyrins. Though earlier reports[7] showed that the second peak has the same width as the first peak, we have observed for the first time in porphyrin dyes and black ink, the second peak as broader than the first one. The second peak moves symmetrically across the main peak with delay of beam 2 and appears exactly at $\tau = |\delta|$. The separation between the two peaks is exactly δ. A constant background appears in all of the profiles.

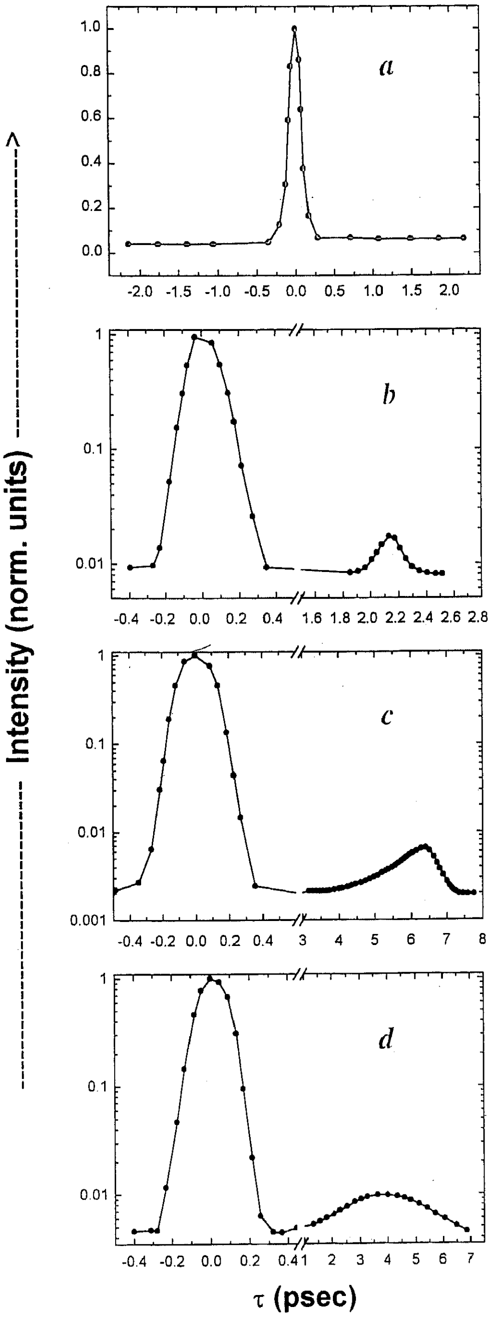

Figure 4. *a*, Signal profile for $\delta = 1$ ps in sample RhB in methanol; *b*, Signal profile for $\delta = 2.1$ ps in sample erythrosinB in methanol; *c*, Signal profile for $\delta = 6.4$ ps in sample black ink in distilled water; *d*, Signal profile for $\delta = 4$ ps in sample CoTTP.

The population relaxation times have been calculated using the relation[5]

$$I_0 : I_\delta : I_b = (1/\gamma_{gg}\tau_c) : 1:1$$

$$T_1 = 1/\gamma_{gg} = (I_0/I_\delta)^*\tau_c.$$

The values of T_1 for various samples are 16 ps in erythrosinB, 26 ps in black ink and 37 ps in CoTTP. There is no decay observed in the coherence peak due to beams 1 and 3 from which one can infer that T_2 of the samples is much less than 160 fs, correlation time of the pulses.

Open aperture Z-scan[10] studies were performed for all the samples to assess their nonlinear absorption. All the samples except RhB show a valley which implies that there is excited state absorption. The samples which showed excited state absorption had two peaks, whereas RhB which shows no excited state absorption, has only one peak. Figure 5 depicts the energy level structure for the organic molecules in general, indicating different transitions. Extensive fs studies on dye molecules revealed the presence of subpicosecond transients which have been attributed to redistribution of excitation energy among the vibrational modes of the molecule[11]. Also reverse saturable absorption in tetra phenyl porphyrins[12], implying that there is excited state absorption, is well established. Our samples also show similar behaviour. The laser pulses excite the molecules from the ground state S_0 into the higher vibrational states of the excited singlet state S_{1v}. The relaxation of the molecules out of this state occurs rapidly into an equilibrium level in the singlet state S_1. This level relaxes either by nonradiative decay into the first triplet state T_1 (K_{ISC}) or by both radiative and nonradiative decay within the singlet system. Depending on the intersystem crossing rates, the excited state absorption may be from singlet S_1 or triplet state T_1 into S_n or T_n respectively. Under steady state conditions, one would expect the population in the S_n as well as T_n levels. Since the pulses have a correlation time of 160 fs, the intersystem crossing rate would not

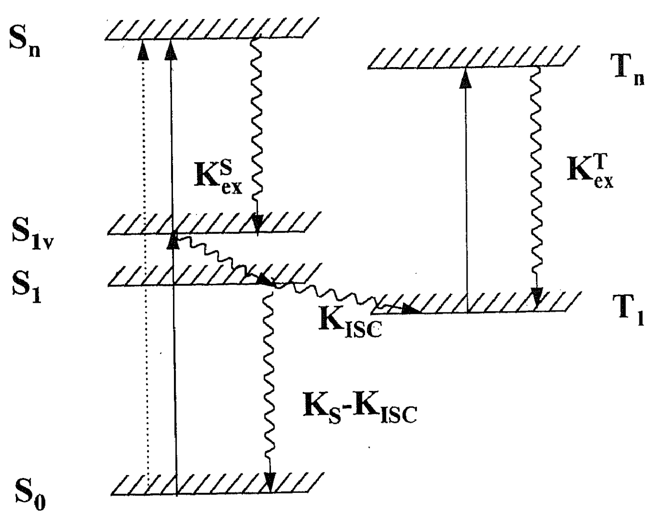

Figure 5. Energy level structure in organic molecules indicating different transitions.

play an important role in these studies. Two photon absorption and the excited state absorption, appearing in the 160 fs time scale, take the molecules from the vibrational manifold to the upper states of S_n. The upper states S_n will relax back to the S_1 state on fs time scale and in the process generate a vibrationally excited state. Peak 1, which has a fast response time in fs, is attributed to the dephasing/relaxations in the excited states S_n. The width of the second peak varies from sample to sample and is attributed to the vibrational relaxation (S_{1v} to S_1) within the molecule. The relaxation time within the vibrational levels, in metalloporphyrins, is reported to be in the order of few ps[13]. The difference in the intensities of the two peaks can be explained as due to the efficiencies of the two gratings. Peak 1 is due to coherence of beams 1 and 3, while beam 2 gets diffracted in $\mathbf{k}_4$ direction and can be imagined as a transmission grating. Whereas the broader peak 2 is due to the coherence of beams 2 and 3 and beam 1 gets diffracted in $\mathbf{k}_4$ direction and can be imagined as a reflection grating. The variation in the widths of the second peak indicates that these systems have different S_1 states dephasing time scales. As the experiments are carried out at room temperature, the 600 nm excitation takes RhB molecules to S_1 (absorbance of 0.01) through vibrational levels of the ground state[14]. Our intensity-dependent studies of the anti-Stokes emission indicate that there is no two-photon absorption for the intensities used in DFWM-IL. As RhB has a dephasing time of the fs order and a longitudinal relaxation of 3 ns, we do not observe the second peak (like in other samples) and the width of the first peak would give us the autocorrelation profile of the pump. The asymmetry in the behaviour of the second peak in Figure 3 *c* could be due to the complex relaxation rate in the ink. A proper analysis of this relaxation phenomenon requires an exact composition of the ink. Further studies are in progress to simulate similar results.

In conclusion, we have estimated the population relaxation times of a few organic dyes using DFWM-IL. When beam 2 delay is non zero, we observe a second peak which is less intense than the coherence peak due to beams 1 & 3. By calculating the intensity ratio of these peaks, we could estimate the population relaxation time. From the width and intensity ratios of the peaks, we could estimate the dephasing times and the longitudinal relaxation rates of the excited states S_n and S_1. We attribute the width of the second peak in erythrosinB, ink and porphyrin to the vibrational relaxation within the singlet state, S_1.

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Micropropagation of two species of *Rauvolfia* (Apocynaceae)

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***Rauvolfia micrantha* – a rare, endemic species in the Western Ghats of India and *R. tetraphylla* – an introduced and naturalized species are economically important for their alkaloids. While tissue culture studies on the relatively more important sarpagandha (*R. serpentina*) are aplenty, the studies in these related species are limited. Proliferation from the axillary bud on the Murashige and Skoog medium, supplemented with 2 mg/l of benzyladenine (BA) induced the quiescent buds within 15 days of inoculation (*R. tetraphylla* – 75% and *R. micrantha* – 82%) and further multiplication of the shoots was best achieved when the medium contained adenine sulphate (0.005 mg/l) and lower BA levels. Rooting of these shoots was possible both *in vitro* (2 mg/l of indole butyric acid (IBA) or *ex vitro* (with 100 mg/l) of IBA) with about the same frequency. Reports on the micropropagation of wild species of *Rauvolfia* indicated low multiplication rates (1:3), while we report here a high rate (1:9) in both spp. (viz. *R. micrantha* and *R. tetraphylla*) and the successful weaning (*ex vitro*) of 60% of such shoots.**

THE genus *Rauvolfia* belonging to the family of Apocynaceae consists of shrubs or undershrubs or occasionally trees distributed in tropical Asia, Africa and America. Five species are recorded in India, including one which is introduced and naturalized (*R. tetraphylla*).

Rauvolfia serpentina (Sarpagandha) is economically important because of the presence of reserpine and rescinnamine group of alkaloids used in the allopathic systems for the treatment of hypertension, cardiovascular diseases and as a sedative tranquilizing agent[1,2]. Although for centuries the drug in its crude form has been used in the Ayurvedic system of medicine in India, realization of its potential has led to its indiscriminate collection coupled with limited cultivation[2,3]. The Government of India restricted the export of crude drug to conserve the natural growth and thus reduce, its exploitation, resulting in the shortage of these alkaloids in the world markets[2]. This led to an active search for these alkaloids in other related species like *Rauvolfia micrantha*, a rare species endemic to the Western Ghats[4,5] and *R. tetraphylla* an introduced and naturalized species, which is very common. However, both are economically important[6] as they contain alkaloids such as ajamalicine, reserpiline, reserpine, sarpagine and serpentine[2]. In many species of this genus, propagation by seeds is not satisfactory owing to highly variable germination rates (10–75%) (ref. 7). Further, parthenocarpy and somatoplast sterility have been reported[2], emphasizing the necessity to develop mass propagation protocols for this genus. Mass propagation in *R. serpentina* was reported by multiple shoot formation[6,8], lateral bud proliferation[9] and somatic embryogenesis[10]. Tissue culture studies on other related species are however, limited.

Plants of each species were collected from their natural habitats (forests of Western Ghats, India), potted and maintained in the greenhouse. Nodal segments were excised from the mature plants of *Rauvolfia micrantha* and *R. tetraphylla*.

The explants were initially washed in 1–2 drops of Tween 20 (10%) per 100 ml followed by rinsing in running water. The surface was sterilized with mercuric chloride (0.1%) for 10 min and then rinsed in sterile distilled water 3–5 times before inoculation into Murashige and Skoog (MS) medium[1]. The pH of the medium was adjusted to 5.8 and solidified with phytagel (0.2%) before autoclaving for 15 min at 1.1 kg/cm^2. The explants were cultured singly in 150×25 mm culture tubes each containing 20 ml medium fitted with cotton plugs. The cultures were maintained at 25 ± 2°C under fluorescent light of about 3000 lux for 16 h per day. For each treatment 20 replicates were used and each experiment was repeated at least thrice. The observations were made at the end of four weeks after incubation.

The nodal segments were cultured on MS medium containing 0.5, 1.0 and 2.0 mg/l of cytokinins, benzyladenine (BA) and kinetin (Kin) individually or in combination. Multiplication of these shoots was tested in these same media or supplemented with adenine sulphate (0.005 mg/l) and IAA (0.5 mg/l). The efficacy of the plant growth regulators was assessed by counting the number of shoots per nodal segment. Individual shoots, which were 2–3 cm long, having at least two leaves and 2–3 nodes, were excised from the shoot clump and transferred to full or half strength MS medium containing IBA (2 mg/l) and with either 3% or 6% sucrose for rooting. Also *ex vitro* rooting was possible by using individual shoots which were 3–4 cm long after dipping in IBA (100 ppm) for 15 seconds. Such shoots were planted in sterile sand and maintained at high humidity (85–90%). After four weeks of root induction, the plantlets were transferred to the greenhouse in small polycups with sterile soil and covered with a plastic bag to maintain humidity.

Establishment of explants *in vitro* was difficult due to excessive exudation of phenolics. Two to three changes of media during the first week of culture were found to be most beneficial for a satisfactory response of explants. Bud sprouting from the nodal explants was observed after two weeks of incubation. Table 1 indicates that with BA (2 mg/l) the explants showed maximum

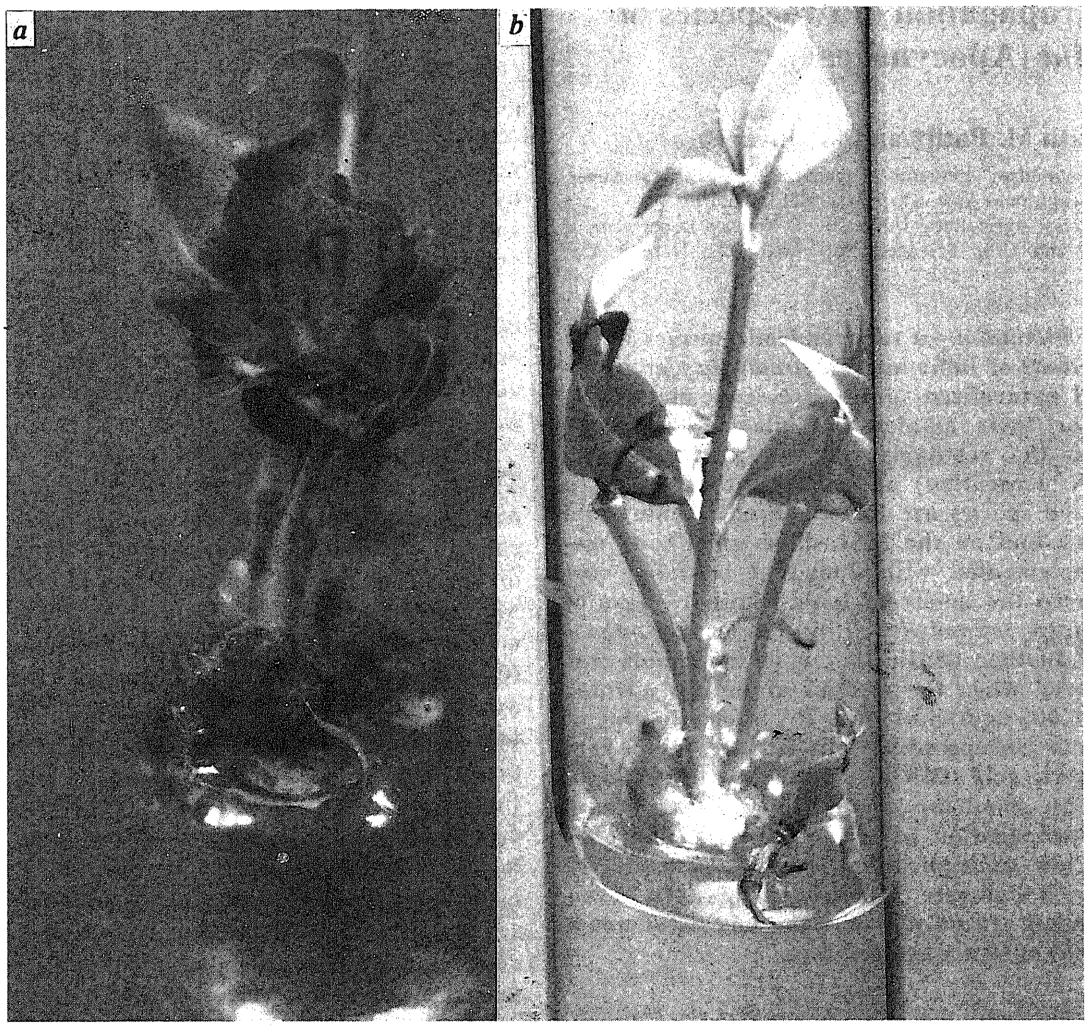

Figure 1. *a*, Multiple shoot formation in *R. tetraphylla*; *b*, Multiple shoot formation in *R. micrantha*.

Table 1. Effect of growth regulators and additives on shoot induction and shoot multiplication from nodal explants of *Rauvolfia tetraphylla* and *Rauvolfia micrantha*

Growth regulators concentration (mg/l) (a)	Shoot induction (%)*		Number of shoots per culture**	
	Rauvolfia tetraphylla	*Rauvolfia micrantha*	*Rauvolfia tetraphylla*	*Rauvolfia micrantha*
BA (0.5)	50	48	4.14 ± 0.23	4.55 ± 0.26
BA (1.0)	55	60	4.35 ± 0.29	4.55 ± 0.31
BA (2.0)	75	82	4.10 ± 0.26	3.90 ± 0.21
BA (0.5) + ADS (0.005)	nd	nd	9.45 ± 0.26	7.77 ± 0.25
BA (1.0) + ADS (0.005)	nd	nd	6.15 ± 0.25	4.55 ± 0.23
BA (1.0) + IAA (0.5)	nd	nd	2.00 ± 0.22	1.95 ± 0.18
KIN (0.5)	48	52	1.15 ± 0.15	1.05 ± 0.12
KIN (1.0)	56	58	2.75 ± 0.24	2.65 ± 0.25
KIN (2.0)	62	58	2.55 ± 0.23	3.40 ± 0.31
KIN (0.5) + ADS (0.005)	nd	nd	2.70 ± 0.21	4.85 ± 0.28
KIN (1.0) + IAA (0.005)	nd	nd	3.35 ± 0.28	1.85 ± 0.20
KIN (1.0) + IAA (0.5)	nd	nd	1.74 ± 0.16	1.75 ± 0.16
BA (0.5) + KIN (0.5)	65	62	4.95 ± 0.34	4.60 ± 0.55

(a) Supplemented in MS medium.
*Average of 20 explants repeated thrice.
**Mean ± standard error (20 replicates repeated thrice).
nd, Indicates not determined.

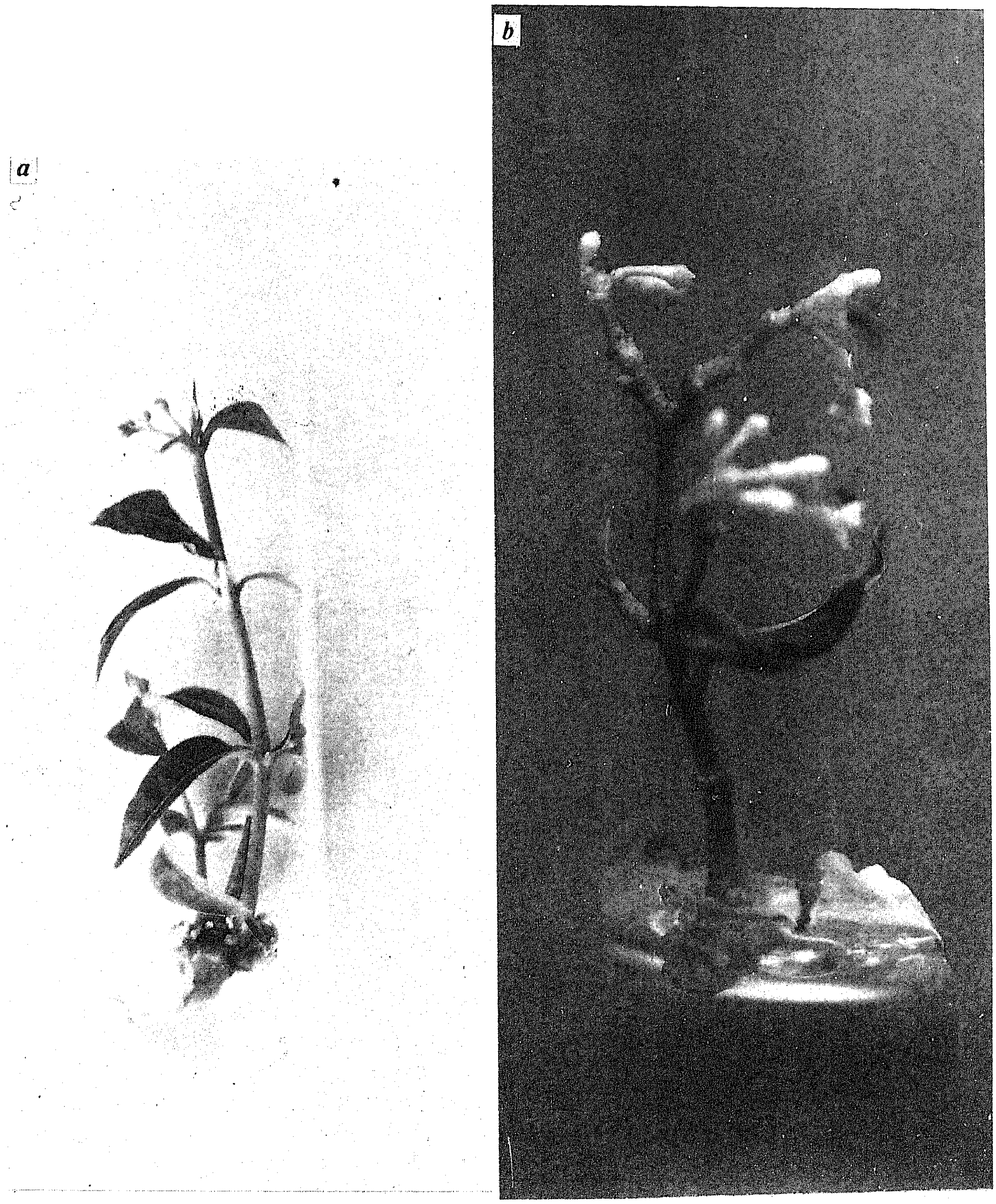

Figure 2. *a*, *In vitro* flowering in *R. tetraphylla*; *b*, *In vitro* flowering in *R. micrantha*.

number of axillary sprouting. BA alone produced better result than Kin alone in both the species; being 75% in *R. tetraphylla* and 82% in *R. micrantha*.

The influence of growth regulators on multiplication of the induced shoots is presented in Table 1. Between BA and Kin, all concentrations of BA tested induced an average of 4 shoots per culture in both the species, while in Kin the number of shoots formed were nearly halved. When BA was used along with Kin, it gave better results (*R. tetraphylla* – 4.95 and *R. micrantha* – 4.60) than using BA or Kin alone. Addition of indoleacetic acid (IAA at 0.5 mg/l) did not help shoot proliferation (Table 1). Instead callusing at the base of explants was seen, which suppressed the growth of the shoot. This was opposed to the reports by Mathur *et al.*[8], who showed that the addition of IAA along with BA helped in higher multiplication of *R. serpentina*. More recently, Sudha and Seeni[12] had also shown this requirement for NAA

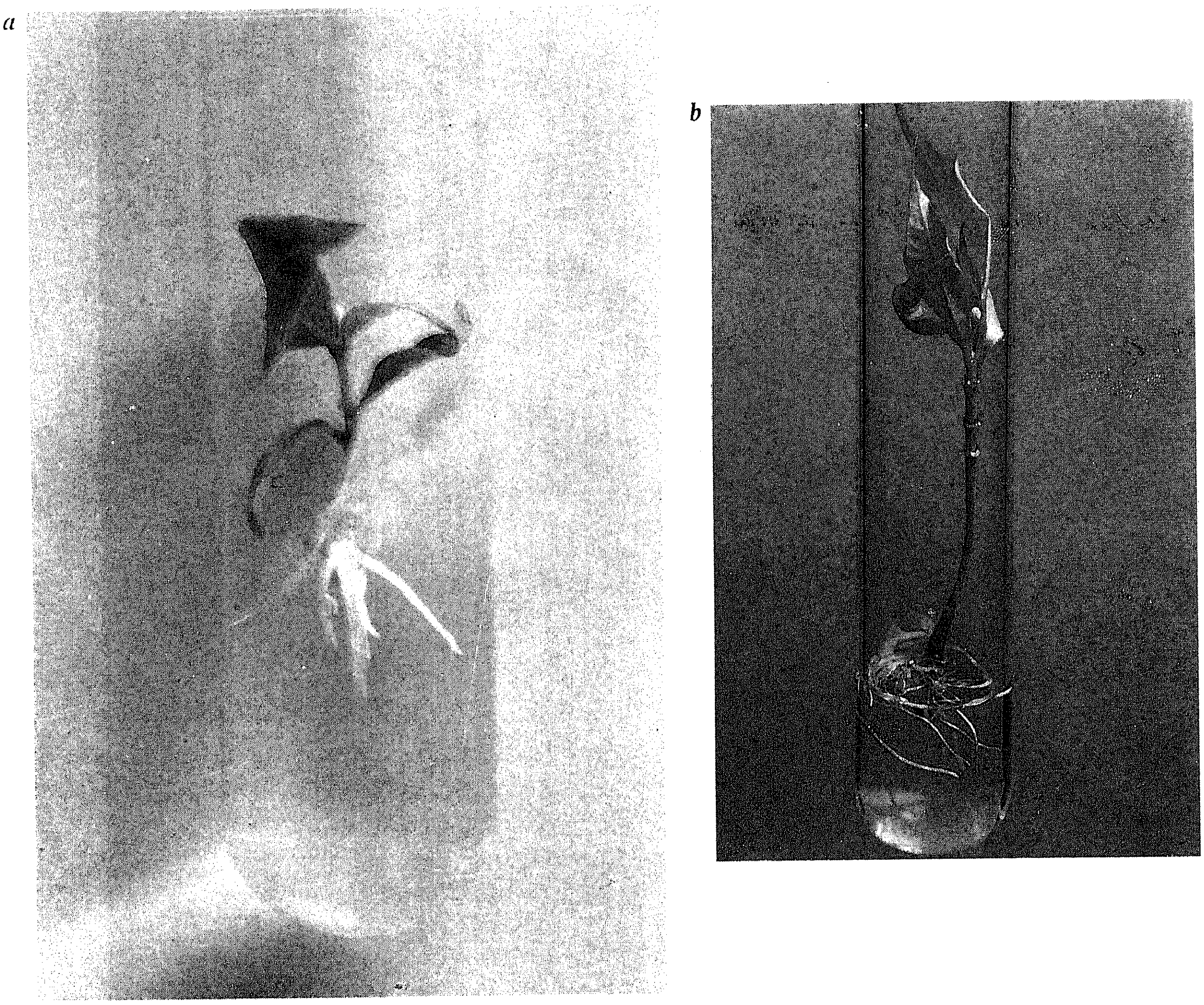

Figure 3. *a*, *In vitro* rooting in *R. tetraphylla*; *b*, *In vitro* rooting in *R. micrantha*.

Table 2. *In vitro* rooting response of the two *Rauvolfia* species

Media combination	Rooting percentage*	
	Rauvolfia tetraphylla	*Rauvolfia micrantha*
MS with 3% sucrose and 0.5 mg/ml of IBA	35	38
0.5 MS with 3% sucrose and 0.5 mg/l of IBA	43	40
0.5 MS with 3% sucrose and 1 mg/l of IBA	42	45
0.5 MS with 4% sucrose and 0.5 mg/l IBA	50	48
0.5 MS with 6% sucrose and 0.5 mg/l of IBA	68	65

*Average of 40 cultures repeated thrice.

during multiplication in *R. micrantha*. These contrasting results may reflect the juvenile nature of their initial explants while we had used explants from old stands (from the forest).

The effect of adenine sulphate on the axillary shoot formation during multiplication was also studied. These results (Table 1) showed that adenine sulphate with BA is the most effective for both species. The number of shoots (*R. tetraphylla* – 9.45 and *R. micrantha* – 7.77) in both the species increased twice more than culturing without adenine sulphate. These results are also three times better than the recent report[13] using BA and NAA for multiplication. In the literature[13], the effective range for adenine sulphate used is 50–250 mg/l. But we are reporting here for the first time that low concentration (0.005 mg/l) was effective for the two *Rauvolfia* species studied here. Although we are not able to fully explain this important observation, the use of high adenine sulphate in other systems (especially for tree species) needs to be reconsidered. Routine subculture of the shoots was in the MS medium containing 1 mg/l BA and 0.005 mg/l of adenine sulphate every four weeks, resulting in high frequency of multiple shoots (5–6 shoots/explant – Figure 1 *a* and *b*). The use of low levels of adenine sulphate may also help in induction of

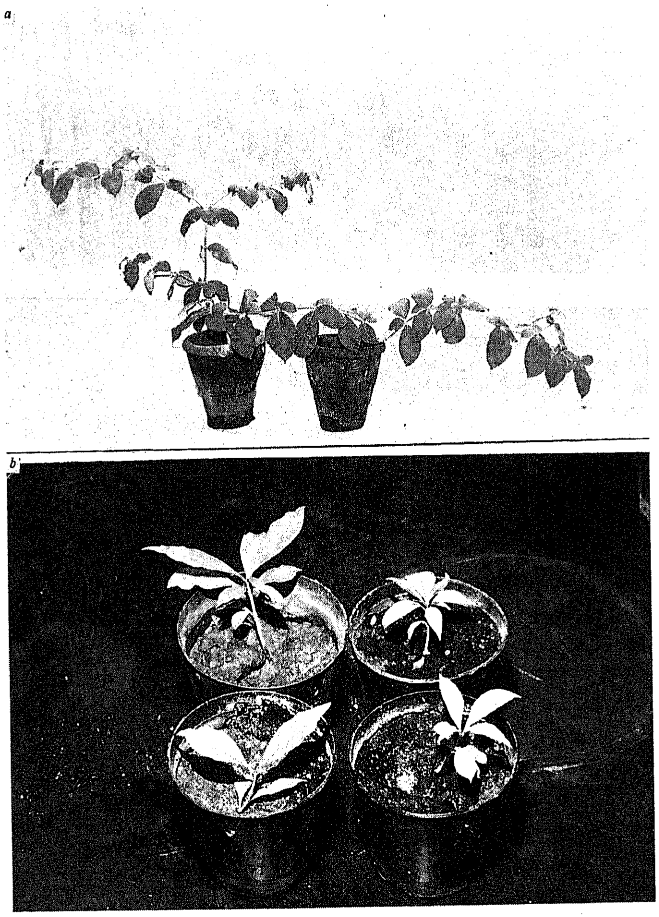

Figure 4. *a*, Potted plants of *R. tetraphylla*; *b*, Potted plants of *R. micrantha*.

in vitro flowering which we observed in 20–40% of these cultures (Figure 2 *a* and *b*).

From proliferated shoot clumps, individual shoots were rooted within two weeks (Figure 3 *a* and *b*) and Table 2 indicates the rooting response in the two species. Roots produced in auxin-free media were thin and the number of roots/cutting were low. Media supplemented with IBA, 2 mg/l improved rooting frequency by 7–8% in both the species. Doubling sucrose in the rooting medium increased the percentage of rooting by 26% in *R. tetraphylla* and 20% in *R. micrantha*. A mortality of 43% was observed during *ex-vitro* rooting. After four weeks the plants were transplanted into pots (Figure 4 *a* and *b*). Plants taken from auxin-supplemented media survived transfer to soil better than those taken from auxin-free media.

The induction of multiple shoots through axillary branching is now recognized as a useful technique for propagation. The present work demonstrates a simple procedure for rapid clonal multiplication of *Rauvolfia* species through axillary branching. The number of shoots formed was best when BA was amended with low adenine sulphate levels. Individual plants may be acclimatized by rooting the shoots *ex vitro*. Thus by this technique, *in vitro* conservation of threatened plants, especially those in which roots contain the active compound[14], is possible.

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Quaternary radiolarian faunal changes in the tropical Indian Ocean: Inferences to paleomonsoonal oscillation of the 10°S hydrographic front

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The northern Indian Ocean is characterized by three distinct surface water masses, i.e. (i) highly saline (> 34.5 ppt) Arabian Sea, (ii) low saline (< 34.5 ppt) Bay of Bengal and (iii) a moderate salinity Indian Ocean watermass south of 10°S hydrographic front. Hence, changes in the surface salinity in this region are index for the paleomonsoon. Radiolaria, the oceanic microplanktons, are extremely sensitive to the

monsoonal salinity, temperature and productivity in the tropical Indian ocean. We refined the earlier radiolarian factor assemblage model by relinquishing the rare groups (< 2% abundance) that caused noise and suppressed the environmental signals. Refined analysis resulted in an additional factor characterizing the 10°S hydrographic front. The 4-factor based radiolarian model exhibited better relationship with the surface hydrographic characteristics of the overlying watermasses. We studied changes in down-core factor values in a sediment core SK-69/1, which has an excellent geological record for the last 0.2–1.4 million years. Down-core factorial fluctuations corroborate to changes in the paleomonsoon. Conspicuous variation in the values of factor-4 (0.2–0.6) characterized the oscillation of 10°S hydrographic front due to changes in the regional oceanic precipitation over the core-site coupled with variations in the monsoonal fresh water debauched by the Indian rivers into the Bay of Bengal. The frontal oscillation indicated an apparent cyclicity of ~ 120,000 years (± 20,000), which corresponds to the ~100,000 years Earth's orbital eccentricity cycle during the Quaternary.

THE Asian monsoonal rainfall is the backbone of the Indian subcontinent and the southeast Asian countries. The paleomonsoonal study may provide information on the behaviour of monsoon in the geological past which may be used for the monsoonal modelling. The present study attempts to record the monsoonal changes in the tropical Indian Ocean using proxy data of radiolarian microplanktons. Although the monsoon is an atmospheric phenomenon, it has a pronounced effect on the surface salinity due to enormous freshwater flux (90×10^3 m^3/s) through the Ganges–Bramhaputra rivers during July–August (peak of SW monsoon) into the Bay of Bengal[1]. However, it is a very conservative estimate because several other rivers [Krishna, Cauvery, Mahanandi, Godavari and Irrawadi (Myanmar)] also discharge huge amounts of freshwater into the Bay of Bengal. Similarly, the southeast Asian rivers (Mikong and Siam, etc.) debauch enormous fresh water into the Indonesian Sea during the SW monsoon. Besides, a regional negative evaporation (minus) precipitation balance (-ve E–P) exists over the northeastern Indian Ocean and the Indonesian Sea[2]. Consequently, the surface salinity of the northeastern Indian Ocean and the Indonesian Sea is considerably lower (< 34.5 ppt) than the oceanic mean (35 ppt). In the western Indian Ocean, however, water remains highly saline (> 34.5 ppt) toward the Arabian Sea which does not receive major freshwater flux (except Indus, Narmada and Tapti rivers) besides having a positive regional evaporation (minus) precipitation (+ve E–P) budget[2]. These two extreme salinity watermasses are separated by an oblique boundary along 34.5 ppt isohaline (Figure 1 *A*) south of Sri Lanka[3]. The isohalines become sub-parallel in areas south of 10°S latitude, representing a hydrographic front that separates northern monsoonal gyre from southern subtropical gyre[3]. Wyrtki[4] described it as the 10°S hydrographic front, a major oceanographic boundary characterized by unique salinity, temperature, oxygen contents from the surface waters to the abyssal depth extending from south of Java to Madagascar. The area is

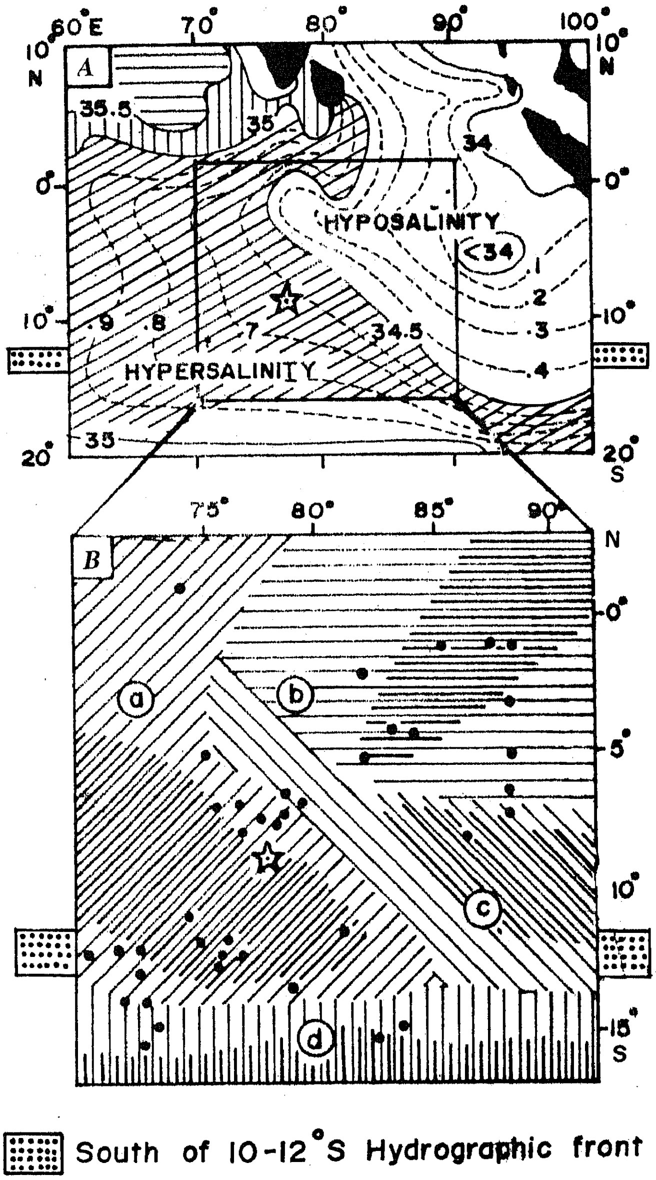

Figure 1. Modern surface salinity during July–August and related radiolarian factors in the central tropical Indian ocean. *A*, Surface salinity during July–August[3] divides the central Indian Ocean obliquely along the 34.5 ppt isohaline into SW hypersaline (>34.5 ppt) and northeastern hyposaline (<34.5 ppt) parts. *B*, Locations of 42 Holocene surface sediments (dots) and four radiolarian factors in the central Indian Basin. Double and single lines depict >0.7 and >0.5 factor loadings respectively. *a*, SW hypersaline factor 1; *b*, Northeast hyposaline factor 2; *c*, Transitional boundary-line factor 3 along 34.5 ppt isohaline south of Sri Lanka[3]; *d*, Southern factor 4 representing Wyrtki's[4] hydrographic front at 10°S. Asterisk mark is the location of sediment core SK-69/1.

Table 1. Sample numbers, latitudes, longitudes, water depth of 42 surface sediments and their respective communnalities and factor loading matrix after varimax rotation

					Factors (assemblages)			
Sample no.	Latitude	Longitude	Water depth	Communality	Southwest (F1)	Northeast (F2)	Transitional (F3)	Southern (F4)
RVG 2483	02.050 S	82.079 E	4590 m	0.86144	–0.47741	–0.65004	–0.44852	0.09898
RVG 2486	05.000 S	82.090 E	4990 m	0.87629	–0.38617	–0.70803	–0.40199	0.25349
RVG 2494	04.000 S	83.190 E	3750 m	0.86572	–0.24726	–0.53067	–0.63079	0.35367
RVG 2501	04.000 S	84.000 E	4860 m	0.89063	–0.29106	–0.82378	–0.16679	0.31539
RVG 2513	01.080 S	85.000 E	4600 m	0.91146	–0.32587	–0.78788	–0.27978	0.32595
RVG 2520	01.125 S	87.020 E	4600 m	0.87957	–0.22177	–0.78658	–0.41872	0.19065
RVG 2528	08.070 S	86.109 E	5200 M	0.89504	–0.26691	–0.27413	–0.82478	0.26151
RVG 2531	07.012 S	88.155 E	5080 m	0.86860	–0.26158	–0.53161	–0.70482	0.14417
RVG 2532	06.001 S	88.090 E	5150 m	0.87537	–0.32955	–0.36418	–0.39331	0.69241
RVG 2533	05.075 S	88.180 E	5030 m	0.83807	–0.34545	–0.60744	–0.43951	0.39571
RVG 2535	03.062 S	88.065 E	4820 m	0.91742	–0.38536	–0.33708	–0.69327	0.41794
RVG 2237	01.059 S	88.095 E	4620 m	0.91334	–0.32799	–0.73511	–0.44973	0.25123
F 47	13.800 S	73.999 E	5150 m	0.93683	–0.72173	–0.33927	–0.47777	0.26939
F 56	14.002 S	73.001 E	4389 m	0.68780	–0.61231	–0.31733	–0.26013	0.38014
F 81	12.490 S	78.011 E	4977 m	0.88063	–0.66523	–0.23442	–0.57234	0.23573
F 99	05.000 S	75.992 E	4492 m	0.79227	–0.43174	–0.67206	–0.20912	0.33240
F 101	07.027 S	76.002 E	5290 m	0.85812	–0.74139	–0.35082	–0.20303	0.37970
F 150	07.644 S	78.954 E	4700 m	0.92248	–0.61965	–0.41068	–0.41431	0.44520
F 151	07.489 S	78.014 E	4750 m	0.90265	–0.53312	–0.52796	–0.48894	0.31722
F 152	07.840 S	77.980 E	NA	0.94388	–0.52815	–0.42385	–0.67137	0.18589
F 153	08.024 S	76.967 E	5000 m	0.92272	–0.77829	–0.20063	–0.51487	0.10794
F 154	07.067 S	76.989 E	NA	0.92586	–0.60898	–0.60671	–0.20632	0.37991
F 155	07.004 S	78.495 E	NA	0.88516	–0.61375	–0.37346	–0.55338	0.25053
F 156	07.067 S	79.500 E	NA	0.87597	–0.61855	–0.26188	–0.53825	0.36754
F 157	06.868 S	77.669 E	NA	0.91415	–0.59119	–0.50440	–0.45089	0.32699
F 158	06.506 S	78.945 E	NA	0.86084	–0.48506	–0.59623	–0.41634	0.31100
F 199	12.337 S	76.377 E	5400 m	0.90312	–0.53190	–0.21895	–0.66120	0.36753
F 379	01.089 N	74.670 E	2475 m	0.80524	–0.45336	–0.38856	–0.47925	0.46803
SS 105	11.010 S	74.985 E	5022 m	0.93133	–0.69447	–0.36103	–0.48079	0.29587
SS 120	13.006 S	72.984 E	4300 m	0.95222	–0.69579	–0.32206	–0.50242	0.33458
SS 121	12.007 S	73.073 E	4430 m	0.91740	–0.69414	–0.51251	–0.37659	0.17630
SS 124	12.017 S	73.000 E	4390 m	0.92241	–0.48775	–0.38600	–0.59912	0.42020
SS 126	14.019 S	72.016 E	4310 m	0.92419	–0.42492	–0.55081	–0.21443	0.62790
SS 127	11.999 S	70.990 E	4750 m	0.92951	–0.59395	–0.50013	–0.42112	0.38635
SS 128	11.969 S	75.465 E	5007 m	0.94471	–0.60484	–0.33927	–0.60922	0.30435
SS 129	12.006 S	76.452 E	5100 m	0.91840	–0.75998	–0.37402	–0.23737	0.38025
SS 139	11.514 S	81.490 E	5085 m	0.94777	–0.70351	–0.39004	–0.33471	0.43436
SS 183	13.510 S	78.979 E	5388 m	0.91224	–0.47417	–0.37737	–0.34235	0.65406
SS 206	15.090 S	83.560 E	NA	0.94385	–0.54120	–0.34848	–0.54670	0.48025
SS 210	15.487 S	82.950 E	5040 m	0.75740	–0.29804	–0.30430	–0.23992	0.72001
SS 231	14.890 S	73.530 E	4900 m	0.91914	–0.51804	–0.36101	–0.47454	0.54338
SS 241	15.500 S	72.991 E	4650 m	0.91468	–0.68373	–0.45363	–0.14932	0.46809
				Percentage of variance	28.89	23.41	21.99	14.86
				Cumulative variance	28.89	52.89	74.29	89.16

influenced by the south equatorial current which has seasonal fluctuations, i.e. minimal during NE monsoon (40×10^6 m^3/s) and maximum during SW monsoon (54×10^6 m^3/s). It transports low salinity water off northwest Australia due to the Indo-Pacific through flow (IPT)[3–4].

Because majority of living radiolaria inhabit the top 100 m of oceanic watermass[5], the International Indian Ocean Expedition (IIOE) hydrographic vertical profiles[3] in the study area from 0 to 300 m watermass suggested that the radiolaria may record changes in surface salinity, temperature and productivity of top ~100 m waters[6]. In the present paper, we have refined radiolarian factor assemblage model of Gupta[7] by relinquishing the rare groups (<2% abundance) that caused noise and suppressed the environmental signals. Percentage data of radiolarian groups[6–8] from the Holocene surface sediments[6–7] were statistically analysed to relate the faunal assemblages with the monsoonal sea-surface salinity (SSS), temperature (SST) and potential primary productivity (PPP). Later, we quantified down-core faunal assemblages to ascertain paleomonsoonal changes in a

Table 2. Correlation matrix between 4 modern radiolarian factors from the Holocene samples and the modern sea surface salinity, productivity and temperature[3,13]

	Factor-1	Factor-2	Factor-3	Factor-4	Sal-NEM	Sal-fall	Sal-SW	Sal-Spr	PPP-NE	PPP-SW	SST-Dec	SST-Fall	SST-Aug	SST-spr
Factor-1	1.0000													
Factor-2	−0.5234	1.0000												
Factor-3	−0.1587	−0.3662	1.0000											
Factor-4	0.1232	0.2330	0.3621	1.0000										
Sal-NEM	−0.4754	0.4955	0.0899	0.2006	1.0000									
Sal-fall	−0.1101	−0.0770	0.2459	0.1240	0.4293	1.0000								
Sal-J-A	−0.7223	0.5170	0.2145	0.2439	0.6601	0.3726	1.0000							
Sal-Spr	0.5621	−0.3566	0.1881	0.3582	−0.1700	0.3321	−0.2698	1.0000						
PPP-NEM	0.0906	−0.2315	0.0203	−0.2060	0.0362	−0.1400	−0.0511	−0.0761	1.0000					
PPP-SWM	−0.7902	0.3157	0.1806	−0.0782	0.3456	0.1626	0.7727	−0.6476	−0.0340	1.0000				
SST-Dec	−0.0448	0.2761	−0.1476	0.0372	0.0576	−0.5499	−0.0074	−0.2586	−0.0024	−0.0346	1.0000			
SST-Fall	0.3042	−0.2260	−0.2417	−0.3244	−0.4406	−0.7487	−0.5518	−0.1213	0.5301	−0.3805	0.3387	1.0000		
SST-Aug	0.5036	−0.4824	−0.0929	−0.3293	−0.4965	−0.3537	−0.6692	0.1844	0.5840	−0.5173	−0.0064	0.8113	1.0000	
SST-spr	0.4060	−0.5513	−0.2646	−0.6339	−0.4772	−0.1201	−0.6707	0.0422	0.3026	−0.3959	−0.1094	0.5111	0.6607	1.0000

Note: Values > 0.489, 0.393, 0.357, 0.304 and 0.257 are significant at 0.001 (99.9%), 0.001 (99%), 0.02 (98%), 0.05 (95%) and 0.1 (90%) levels respectively. Spring = May, June and July, Fall = Nov, Dec. and Jan (Levitus[13]).

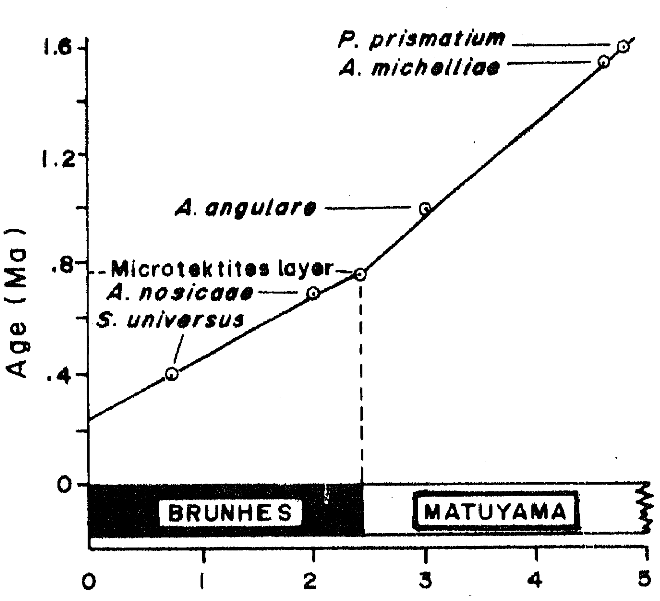

Figure 2. Stratigraphic control of SK-69/1 core bracketed by radiolarian extinction levels[9,17] and Australasian microtektite maxima[14] near Brunhes/Matuyama boundary[15,16]. The first 200 ka record was not recovered.

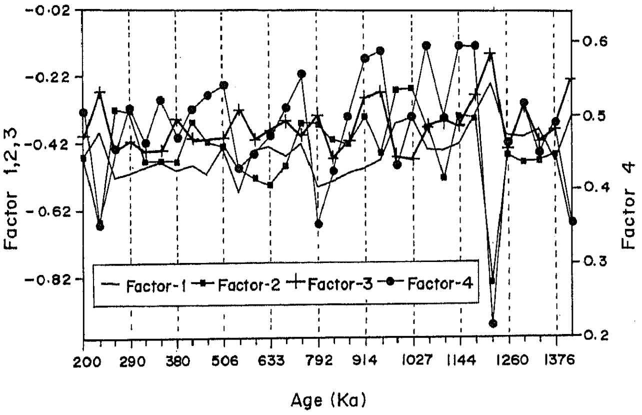

Figure 3. Down-core fluctuation in the radiolarian paleofactors in a Quaternary sediment core SK-69/1. Paleofactors, 1, 2 and 3 oscillated within −0.72 to −0.15, whereas factor 4 oscillated within 0.2 to 0.6 factor values at the core site in the last 200–1400 ka. Approximately 10-peaks (in 1.2 Myrs) of factor-4 suggested oscillation of Wyrtki's[4] 10°S hydrographic front due to changes in monsoonal freshwater flux apparently at 120,000 yrs (± 20,000) cycle in the Quaternary tropical Indian ocean.

sediment core (SK-69/1), which has an excellent geological record for the last ~200,000–1,400,000 years (Quaternary) in the central tropical Indian Ocean.

Based on the sedimentation rate of ~1 cm/1000 years [1000 years = kiloyears (ka)] for the top sections of VM-34-53 (0–236 cm = 210 ka) and RC-14-22 (0–100 cm = 120 ka) sediment cores[9], the 42 surface sediments (top 2–3 cm of the sea floor) from the central Indian Basin represented the Holocene material (Figure 1 *B*). Gupta[6,7] quantified 47 radiolarian groups[8] from these Holocene samples following the random settling method of Moore[10]. Out of the forty-seven, 22 groups contributed <2% abundance and added to the random noise in earlier analysis[7]. In the present analysis, 25 groups qualified the standard criteria of Imbrie and Kipp[11], i.e. (i) relinquishing of groups contributing <2% which only cause the noise, (ii) surface water dwellers and (iii) modern analogues of the fossil groups. Percentage data of 25 groups were analysed by Imbrie and Kipp's[11] method for Q-mode factors, which resolved four varimax factors accounting for 89.15% of distributional variance (v) explained (Table 1). Communality value ($h^2 > 0.89$), which is the internal check for the distortion of data due to differential dissolution, taxonomic inadequacy or the local ecological anomalies, is sufficiently higher than the Imbrie *et al.*'s[12] threshold (0.8) for the meaningful interpretations. The first factor ($v = 28.88\%$, dominant groups *Artiscin* and *Pyloniids*) with highest loading in the southwestern area [Figure

1 *B*(a)] is related with hypersaline (> 34.5 ppt) watermass (Figure 1 *A*). The second factor ($v = 23.41\%$, dominant groups *Spongaster* and Spongodiscids) with highest loading in the northeastern area [Figure 1 *B*(b)] is related to low salinity (<34.5 ppt) watermass (Figure 1 *A*). The third factor ($v = 21.98\%$, dominant groups *Disolenia* and *Collosphaera*) is a transitional assemblage [Figure 1 *B*(c)] characterizing boundary-line salinity condition along the oblique 34.5 ppt isohaline south of Sri Lanka (Figure 1 *A*). The fourth factor ($v = 14.86\%$, dominant groups *Lamprocyclas* and *Anthocyrtidium*) exhibited highest loading [Figure 1 *B*(d)] south of 10N°–12° S low salinity hydrographic front of Wyrtki[4]. The factor loading values were computed for correlation (Table 2) with modern surface salinity, SST and productivity by digitizing oceanographic charts of Wyrtki[3] and the seasonal oceanographic digital data of Levitus[13] on magnetic tapes at respective sampling locations. All factors are correlated well only with the SW monsoonal salinity, SST and PPP of the tropical Indian Ocean (Table 2) and concur with earlier study[7]. Results suggested that these factors, when studied in down-core, may provide insight into paleomonsoonal salinity, SST and PPP changes in the geological past.

Therefore, the down-core radiolarian factors (faunal assemblages) were studied in a sediment core SK-69/1 (Lat. 8.55°S, Long. 77°E, water depth 5388 m, Figure 1 *B*) to decipher monsoonal changes in the geological past. Recently, Shyamprasad and Sudhakar[14] encountered an Australasian microtektite (the melt-splash out fall after the collision of a meteorite on the Earth) maximum at 235 cm core-depth of SK-69/1. Earlier, Schneider *et al.*[15] reported such microtektite maximum to occur at around the Brunhes–Matuyama (B/M) geomagnetic reversal boundary, which is isotopically dated by Baksi *et al.*[16] at ~780 ka before present. Other stratigraphic controls of the sediment core are based on the identification of the extinction levels of *Stylatractus universus* (425 ka)[17], *Anthocyrtidium nosicaae* (700 ka), *Anthocyrtidium angulare* (1000 ka), *Anthocyrtidium michellinae* (1500 ka) and *Pterocanium prismatium* (1600 ka)[9] in the core (Figure 2). Based on these datum levels, the sedimentation rates of Brunhes and Matuyama periods were calculated by plotting datum level vs core depth (Figure 2). As the sedimentation rates of the Brunhes (4.2 mm/ka) and Matuyama (2.8 mm/ka) have a ratio of 3:2, we selected subsamples at 15 cm and 10 cm intervals respectively to maintain regular age-spacing [delta-*t* ~39.5-ka ± 8-ka] in down-core data on radiolarian faunal assemblages. The percentage data on down-core radiolarian fauna were analysed for Q-mode factors by the same method used for the Holocene samples in the present study. Fluctuations in the down-core factor values (paleo-factors) suggested paleomonsoonal changes in salinity, SST and PPP with respect to core depth (Figure 3) as per their relationship in the Table 2. However, troughs of the factor-4 indicated higher freshwater flux during warmer periods, resulting in southward migration of 10°S hydrographic front. Whereas, peaks of the factor-4 suggested less freshwater flux during colder periods, resulting in northward migration of 10°S hydrographic front in geological past. The results suggested that the 10°S hydrographic front has oscillated back and forth almost 10 times (10 peaks of factor 4, Figure 3) from its present position in 1.2 million years, suggesting an apparent cyclicity of ~120 ka (± 20 ka). Such ~100 ka cycle is a prominent feature in excellently preserved geological records[18–20], and it corresponds to the Earth's orbital eccentricity cycle[21]. Therefore, the 120 ka cycle apparently present in the oscillation of the 10°S hydrographic front is due to changes in the surface salinity at the core site. Recently, from the same core data-set, the paleo-sea surface temperatures reconstructed by the radiolarian transfer functions are also reported to oscillate cyclically at ~100 ka, corresponding to the Earth's orbital eccentricity cycles[22].

Obviously our core data have recorded the changes in the surface salinity over the core site (Figures 1 and 3) in the last 1.4 million years. These changes are possible by two mechanisms, i.e. (i) the regional precipitation over the central Indian Ocean coupled with monsoonal freshwater debauched by the Indian rivers into the Bay of Bengal; and (ii) the low salinity surface (0–100 m) and deep (>100–800 m) waters from the Indo-Pacific through flow (IPT) via deeper inner-ocean passages (depth range 70–1000 m) near Bali, Lombok, Savu and Timor Islands[23]. We analysed both possibilities to evaluate the sources of low salinity at core site. Figure 4 *a* depicts the rainfall over the Indo-Pacific region during July based on GOES precipitation index[24]. The oblique precipitation contours over the central Indian Ocean (Figure 4 *a*) are in conformation with the oblique 34.5 ppt isohaline shown in Figure 1 *a*, suggesting the salinity lowering at core-site is the consequence of the regional oceanic monsoonal precipitation coupled with the freshwater debauched into the Bay of Bengal by the Indian rivers. The mechanism of second possibility of IPT is related to the monsoonal winds, pressure gradient and surface currents in the Indo-Pacific region[25]. Surface water flows from the Java Sea and Pacific Ocean into the Banda Sea, causing an accumulation of warm low salinity water and a depressed thermocline due to unfavourable winds which are unable to move it into the Indian Ocean during NE monsoon. During SW monsoon strong southeast winds blow over the region, forming south equatorial current in the Indian Ocean between Java and Australia, generating strong southward flow of upper 200 m water towards Indian Ocean[25]. Because low salinity IPT surface water gushes towards Indian Ocean due to south-east winds, pressure gradient and the formation of south

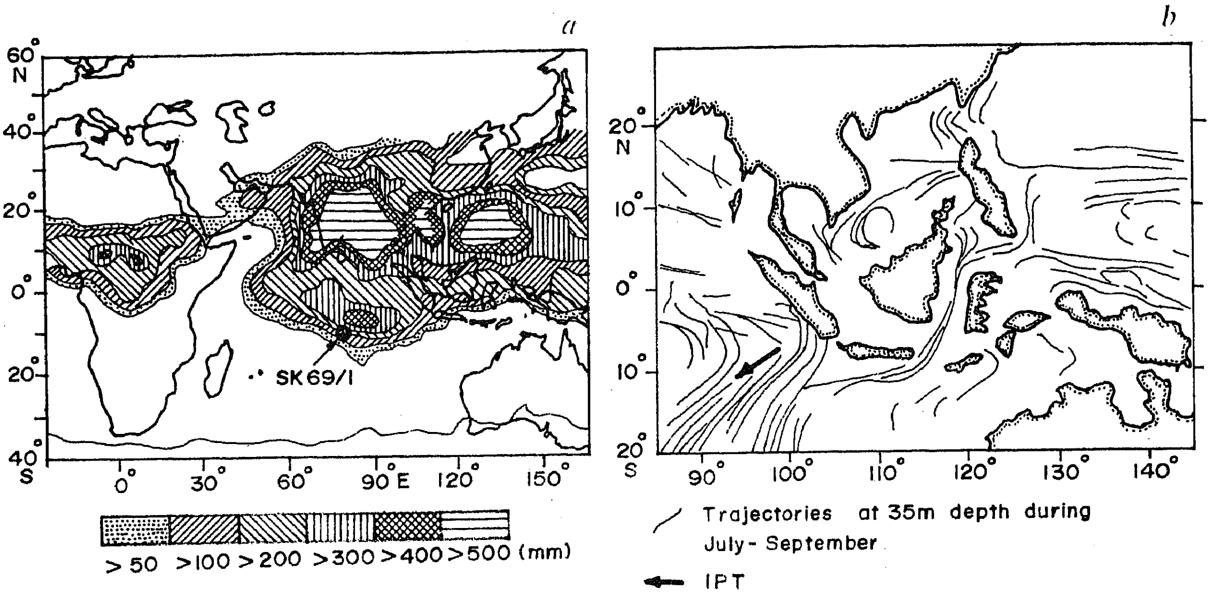

Figure 4. Possible sources of the freshwater causing low salinity at core site. *a*, The precipitation over the Indo-Pacific region showing that the core site is well within 200 mm rainfall during July[24]; *b*, The trajectories of the tracer particles released at 35 m water[23] to trace the Indo-Pacific through flow during SW monsoon showing that SW flowing IPT surface water affected areas beyond 15°S.

equatorial current, the possibility remains that some amount of IPT water might be reaching up to the core site. The trajectories of tracer particles, released at 35 m during different seasons, indicated that the IPT is very seasonal. During SW monsoon the trajectories are in SW direction (Figure 4 *b*) influencing the areas further south than the 10°S (Miyama *et al.*[23], Figures 11 *a–d*, Figures 18 *a*, *b*). Therefore, we surmised that surface IPT water has not much influence on the surface watermass overlying our core site.

However, the possibility of IPT water advected from the sub-surface layers is not ruled out. Therefore, we summarized the vertical salinity profiles of the IIOE[3] during SW monsoon along the east–west transect at the equator, and north–south transects at 70°, 77°, 92° and 110°E longitudes from 0 to 15°S latitudes (Figure 5, index map). The vertical salinity structure (VSS) along equator from 70 to 87°E suggested that low salinity surface water (<100 m) floats over high salinity deep water (> 100 m) during SW monsoon (Figure 5 *a*). The VSS along 70°E from 0 to 17°S opined a low-salinity surface water (<34.8 ppt) down south up to 12°S, and a protrusion of low-salinity deep water of IPT origin below ~400 m (stippled, Figure 5 *b*). The VSS along 77°E from 5°N to 12°S showed low-salinity surface water (<100 m) and a deep low-salinity water of IPT origin (stippled) below 400 m (Figure 5 *c*). The VSS along 92°E indicated a low-salinity surface water (<100 m) and the intrusion of low-salinity deep water of IPT origin (from depth >700 to >100 m beneath the 10°S, Figure 5 *d*). The columnar advection of the low-salinity water of IPT origin (salinity 34.8 ppt, Fine[26], Table 1) represents the 10°S hydrographic front influenced by the IPT. The advection of low-salinity IPT from the deep is more pronounced in the eastern profile along 110°E from 10° to 25°S (Figure 5 *e*), which shows that low-salinity IPT water is almost inseparable from the surface low-salinity water (<100 m). The deeper low-salinity IPT water (<34.8 ppt) is easily recognizable from surface low-salinity watermass due to the regional precipitation coupled with freshwater flux into the Bay of Bengal towards west of 90°E (Figures 5 *b*, *c*). It becomes inseparable from the surface low-salinity water towards east (Figures 5 *d*, *e*). The surface low-salinity water of monsoonal origin (Figures 5 *b–d*) is distinctly due to the regional oceanic precipitation over the central Indian Ocean coupled with the freshwater flux from the Indian rivers into the Bay of Bengal, as it floats over the high-salinity subsurface water (> 100 m). This surface water is intruded by low-salinity deep water of IPT origin towards east of the 90°E (stippled columns, Figures 5 *b–e*). It suggested that fluctuations recorded by surface dwelling radiolarians (<100 m) in our core were not influenced by the intrusion of deep low-salinity IPT water at the core site.

In view of the above, the regional precipitation over the core site, the freshwater debauched by the Indian rivers into the Bay of Bengal and surface IPT occur during SW monsoon. These facts converge to our reasoning that the 100 ka cyclicity recorded in factor-4 is

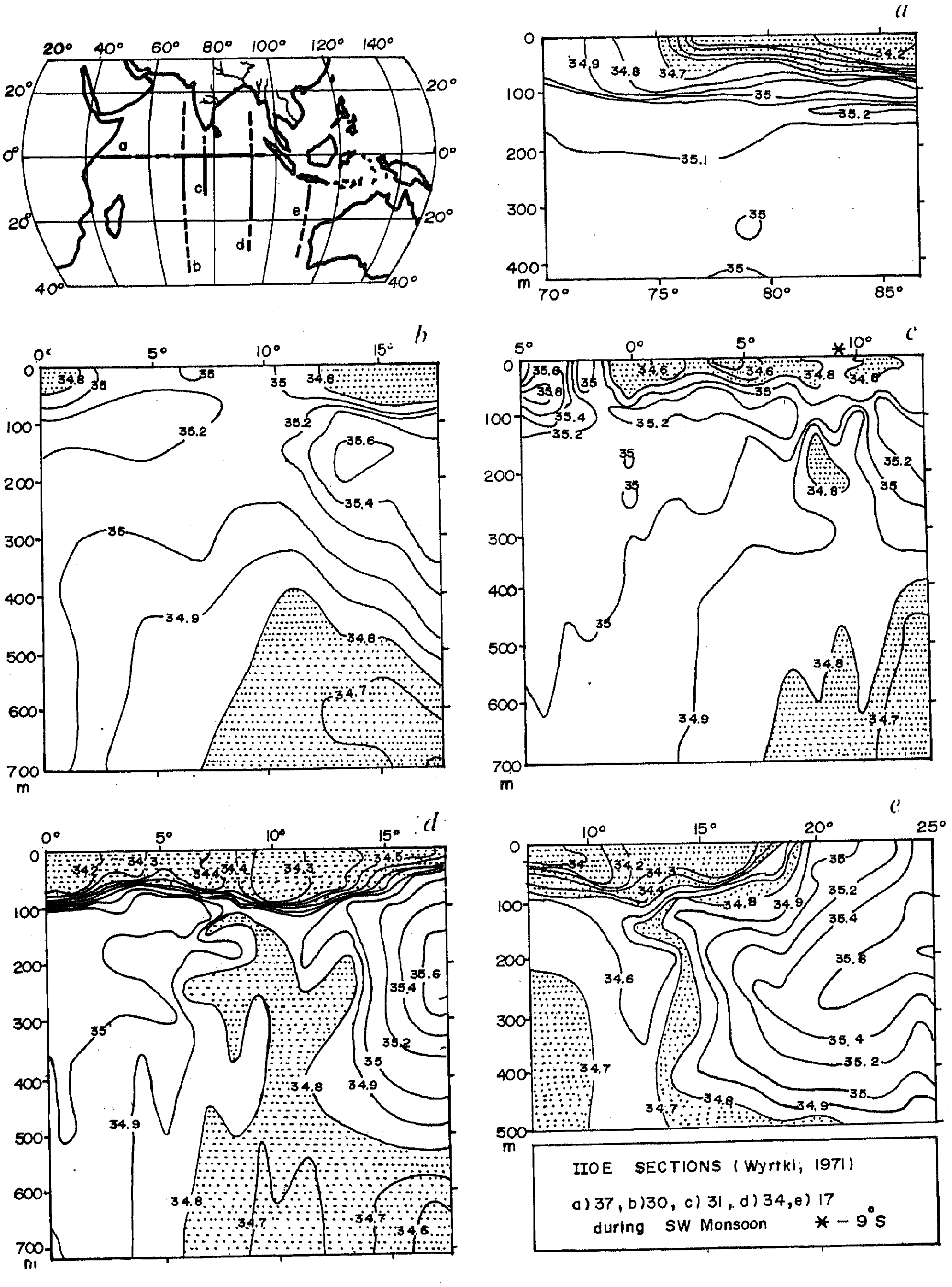

Figure 5. Vertical salinity structures in the equatorial Indian Ocean during SW monsoon. Index map showing the IIOE hydrographic profiles along the equator *a*, 70°E *b*, 77°E *c*, 92°E *d*, and 110°E *e* longitudes. Stippled area denotes low-salinity water masses at the surface (<100 m) due to monsoonal precipitation and deep advecting vertical columns of low salinity (> 400 m, 34.8 ppt[26]) representing the Indo-Pacific through flow[3].

related to the monsoonal fluctuation of the 10°S hydrographic front. The results suggested that radiolarian faunal assemblages in down-core exhibit the boundary-line monsoonal changes in surface salinity of the central Indian Ocean for the last 0.2–1.4 million years. The paleofactor values have potential for the reconstruction of sea-surface salinity, using transfer function technique[11,22,27,28]. Our ~0.2–1.4 million years monsoonal record is larger than 350 ka paleomonsoonal record based on eolian dust flux from Arabian desert[29] to the Indian Ocean. Though results suggested that 10°S hydrographic front has oscillated due to monsoon with

~120 ka eccentricity cycle, its verification requires a high-resolution study because strength of monsoon is strongly affected by the precession (23 ka) and obliquity (41 ka) cycles[30] than the eccentricity cycle (100 ka, range ~95–135 ka). Our results of ~120 ka orbital eccentricity cycles related monsoonal oscillation in the 10°S hydrographic front open an unorthodox view that even though the amplitude of insolation related to the orbital, eccentricity cycles (~100 ka) may be low (<0.1%), compared to precession (23 ka) and the obliquity (41 ka) cycles[31], its modulating effect on the Asian monsoon system cannot be denied and ignored.

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Occurrence of Fe, Mn-rich layer in the distal Bengal fan

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Dark tan-coloured Fe, Mn-rich stiff layer was observed at 45–50 cm and 40–42 cm levels in the Sagar Kanya 72/1 and 100/6 sediment cores respectively, which were collected from distal Bengal fan under BOBCRUST programme. SK 72/1 and SK 100/6 cores were analysed for Fe, Mn, Ni, Co, Cr, $CaCO_3$ and organic carbon (OC) contents. A remarkable increase in Fe, Mn and drastic decrease in $CaCO_3$, OC, compared to overlying and underlying sediment layers was observed at this stiff layer. Change of these elements concentration correlates with the change of fauna from the cold assemblage to warm assemblage. Increase of Fe, Mn, Ni, Co and decrease of Cr, $CaCO_3$, OC indicate that this layer is formed due to post-depositional migration of these elements during the Pleistocene/Holocene transitional period, because of emasculated influence of terrestrial input into these sites.

BENGAL fan is the world's largest submarine fan, which is positioned under the highest run-off receiving bay, and it is built of terrigenous sediments carried from the Himalayan region by the Ganges, Brahmaputra, Irrawaddy and Salween river systems[1,2]. Variations in the run-off are dependent on the monsoons and the waning and waxing of glaciers in the catchment region. The sediment input is mainly controlled by a number of factors such as turbidity currents, sediment flow or fluvial input to the sea, giving rise to these thick fan sediments. Geochemical character of the sediment column reveals such variations and helps in identifying metal sources apart from providing a clue to their removal mechanism from the overlying water column[3,4]. Studies on Bengal fan deep sea cores are very few[5–10], published geochemical

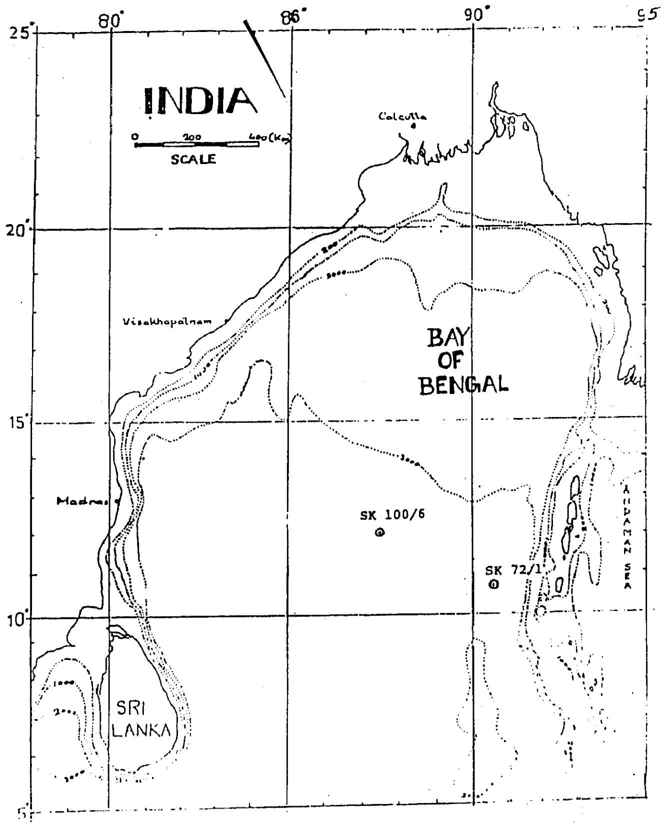

Figure 1. Core location map.

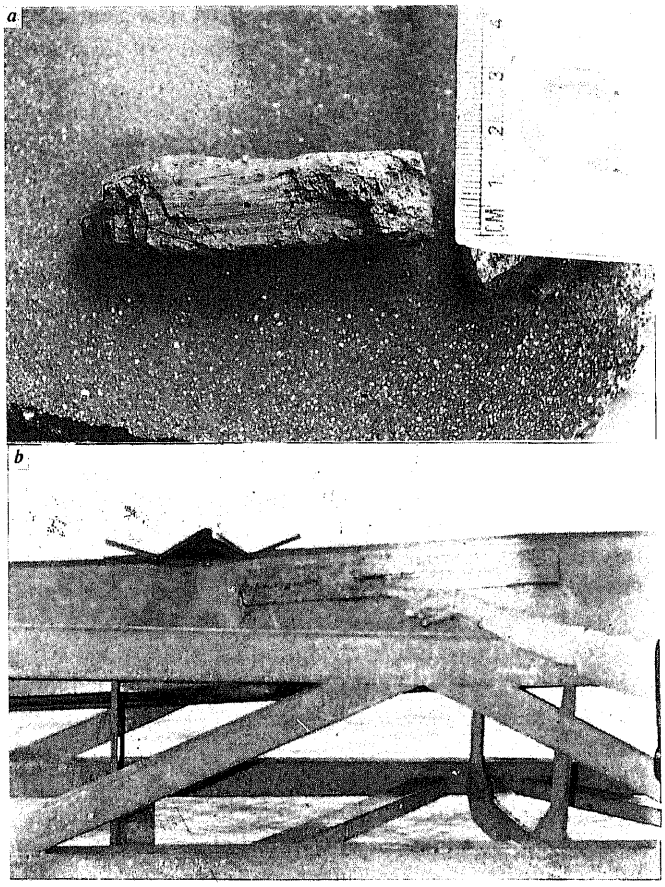

Figure 2. *a*, Stiff layer collected from SK 100/6; *b*, Bend in box corer.

studies are still more scanty. Fe, Mn, enrichment is reported in the Central Indian Ocean[11–14] and surface sediments of the Bengal Fan[5]. The present work describes the occurrence of Fe, Mn-rich layer in two cores collected from distal Bengal fan (Figure 1), with reference to variations in Ni, Co, Cr, $CaCO_3$, OC and the formation of this layer at Pleistocene/Holocene boundary is emphasized.

The concentration of various elements in the bulk sediments was determined by Phillips X-ray fluorescence spectrophotometer, precision and bias (± 5%) were monitored by replicate analyses of selected samples. Organic carbon[15] was estimated to a precision of ± 0.01%. $CaCO_3$ was estimated by standard titration method (± 0.5%)[16]. Colour logs were prepared with Munsell soil colour charts.

Dark tan-coloured, laminated and stiff layer is observed in SK 72/1 and SK 100/6 at 45–50 cm and 40–42 cm levels of the core sediments respectively. The thickness and stiffness of this layer, within the respective sub-sample, vary in both the sites. It is 4 mm in the eastern core (SK 72/1) and 15 mm in the core from Central part of the distal fan (SK 100/6, Figure 2 *a*). The impenetrability and bent of the box corer was thought to be due to this hard layer (on-board) observation SK 100 (Figure 2 *b*). However, this needs to be confirmed after contemplating other mechanical problems involved in it. This hard layer separates the total core mainly into two parts, i.e. light-coloured upper part [yellowish brown (2.5Y/5/4); yellowish gray (5Y 7/2)] and dark-coloured lower part [greenish black (5G/2/1/); olive gray (5Y/5/2)]. It is sandwiched in between these two geochemically different environments.

Iron concentrations in the core sediments vary from 2.5 to 9.9%, 2.78 to 10.27% in SK 72/1 and SK 100/6 respectively. In both the sites, the uppermost part of the core represents lower values, whereas the maximum concentration is encountered at hard layer. Iron values of this layer are 9.9% in SK 72/1, 10.27% in SK 100/6. Along with iron values Mn, Ni and Co, concentrations at these hard layers are conspicuously high. In contrast to this, $CaCO_3$ and OC values are considerably low at this depth. Average concentrations of $CaCO_3$ are 15.46% and 6.27% in the upper and lower parts of the core in SK 72/1 and similarly, 43.33%, 6% in SK 100/6. On an average, organic carbon shows lower values in the upper part and higher values in the lower part in both the cores. Whereas lowest values of $CaCO_3$ (1%; 0.5%) and lowest OC (0.4%; 0.04%) in SK 72/1, SK 100/6 are noticed respectively at the stiff layer. A few centimeters above and below to this layer, abundancy of fragmented foraminiferal shells are noticed.

The antipathetic relationship between $CaCO_3$ and OC indicates that the deposition of these two is not controlled by the same process. It is observed that >63 µm fraction of the sediment consists mainly of foraminiferal and radiolarian shells and their fragments. Hence the coarse fraction is biogenic. It is well established that in marine sediments, $CaCO_3$ being biogenic/authegenic shows an inverse relationship with terreginous material[8,17]. OC in the present site is mainly of terrestrial origin[6], higher concentrations of this terrestrial OC and low $CaCO_3$ indicate the strength of terrestrial input. Upper part of these two core sediments depicts weakening of the terrestrial influence. As an immediate response to the last glacial maxima followed by strong aridity and fast recession of the mouth of the rivers due to retreat of the sea level, the resulting reduction was in the supply of the terrigenous sediments in the Bay of Bengal. It is reflected by the relative change in the $CaCO_3$ and OC at 45–50 cm and 40–42 cm in SK 72/1 and SK 100/6 respectively. Increase in the relative abundance of *Globorotalia menardii*, which is widely used to denote Pleistocene/Holocene boundary, was observed from these depths, substantiating the above argument[18,19]. Similar observations of Mn enrichment at surface and subsurface levels of sediment cores from pelagic environment have been documented earlier[3,20,21]. It has been suggested that in hemipelagic sediments, the onset of green colouration corresponds to the point below which solid phase Fe^{+3} is reduced and pore water Fe^{+2} is present[22]. Depth position of this Fe, Mn-rich layer, clearly indicates that this layer overlies the dark colour reduced sediment column.

As early as 1895, it was suggested that post-depositional remobilization and reprecipitation were responsible for the higher values of Mn in deep sea sediments[23]. Diagenetic processes, such as the post depositional migration of Fe, Mn and other trace elements as a result of a moving redox front, are well known[24–28]. Mn is highly insoluble in oxic environments and is buried as oxyhydroxide particles and coatings in the sediment. In hemipelagic environments, oxidation of labile organic matter causes the sediment to become anoxic at depths and Mn^{++} is mobilized either by the reduction of manganese hydroxides or as a result of solubility equilibria. Manganese then diffuses along a pore water concentration gradient from anoxic to oxic conditions and is immobilized by the oxidation of Mn^{++} and the precipitation of the manganese oxyhydroxides[24,25]. This process can produce near-surface diagenetic manganese contents of several weight percent[29–31]. The behaviour of Fe in marine sediments is similar to that of Mn, with iron hydroxides being mobilized as Fe^{++}, following the utilization of labile manganese oxides[24,25]. Mn responds sensitively to paleo-oceanographic changes. Mn enrichment due to changes in the concentration of the overlying water column mainly because of paleoclimatic events like changes associated with phases of transition from glacial to interglacial periods have been reported for the sediments of the Atlantic and Pacific regions.

The behaviour of Co and Ni, during remobilization, is closely related to that of Mn[25,30,32–34]. Pore water data from hemipelagic sediments[35] indicate that both Co and Ni reach dissolved maxima in the Mn reduced zone and are precipitated along with manganese oxide zone[26–29]. Chromium is definitely depleted in the upper oxidized section of the core, contrary to the case of Mn, Ni, and Co. Cr probably exists in the oxidized mud prevalently as CrO_4^{-2}, which is highly soluble[36]. In the reduced down section of the core, Cr^{6+} will be reduced to Cr^{4+} and Cr^{3+} and precipitated.

Geochemical nature of the sediments in terms of the above described elements clearly envisages (Figures 3, 4) that in the present study, the stiff layer is positioned in between reduced dark colour and oxidized light colour sections of the core sediments.

Organic carbon minima is correlated with Mn maxima, this inverse relationship in deep sea is well established especially at glacial ends, where oxidation is the main process responsible for this lower OC and higher Mn concentrations. Oxidation of organic carbon results in the dissolution of foraminifera[24]. In agreement to this, lowest values of $CaCO_3$ at this stiff layer are due to $CaCO_3$ dissolution at this depth. Fragmentation of foraminiferal shells, slightly above and below this depth, indicates the influence of the dissolution and complete absence of foraminiferal shells at stiff layer is an indication of the intensified dissolution, presence of siliceous fauna such as radiolarians confirm the dissolution of $CaCO_3$. However, such enrichment of metals can also be possible in volcanogenic sediments, but absence of volcanic shards rules out the intuition of volcanic origin of this layer and supports the dissolution phenomena. In general, lamination is one of the sedimentary structures indicating the sedimentation pattern, while laminae in the stiff layer could be considered as micro-level sedimentation during the formation of the stiff layer.

Petrological and XRD investigations were conducted to identify minerals of the above-said metals. As these studies do not show any mineralogical form, this sample is further subjected to SEM studies. These studies indicate that the above-discussed metal concentrations in the stiff layer are not in well-defined crystalline form, they are either in cryptocrystalline form or in the oxide coatings on the sediment particles, which are deposited earlier to the formation of this stiff layer.

Published results of textural and geochemical studies on SK 31/11 core sediments indicate the Pleistocene/Holocene Boundary at 40–45 cm bsf of the core[8]. In the

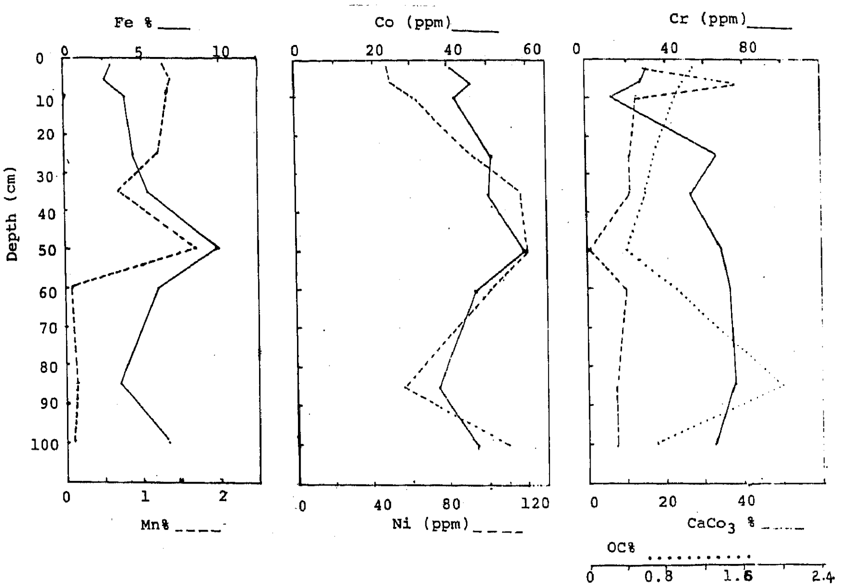

Figure 3. Vertical geochemical variations of SK 72/1.

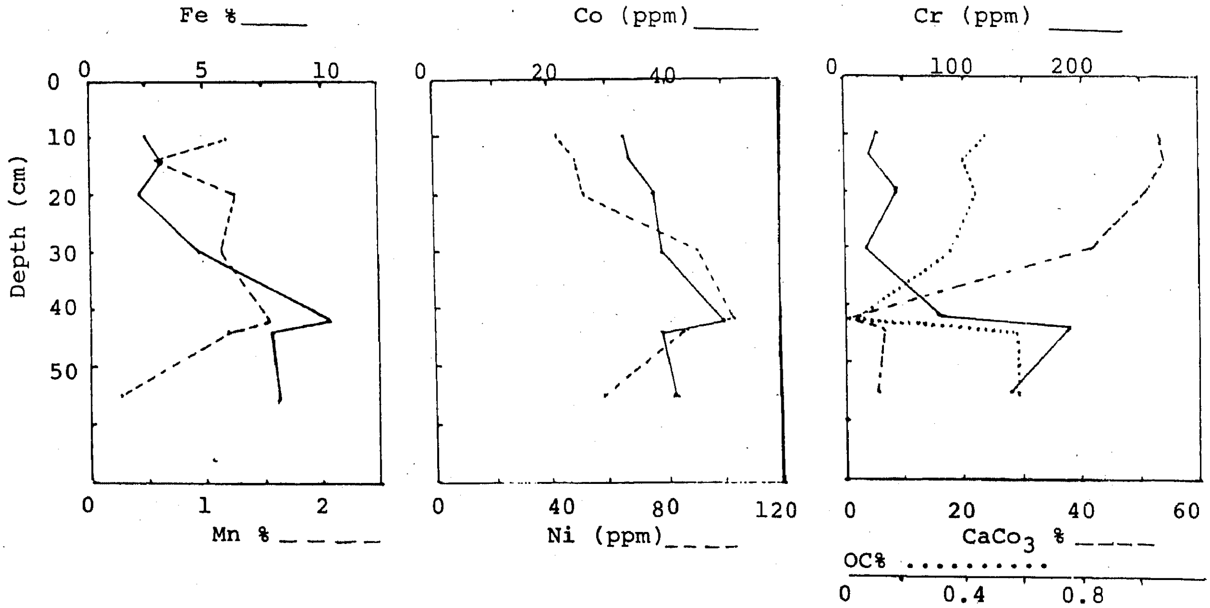

Figure 4. Vertical geochemical variations of SK 100/6.

present study a 7 mm thick Fe, Mn-rich layer is observed in this core also, at the above boundary level. Distributional pattern of geochemical elements in the Holocene and Pleistocene parts of the above core sediments, coincides with our data. It is observed that the thickness of stiff layer increases in SK 100/6 (15 mm) from SK 31/11 (7 mm) and SK 72 (4 mm), indicating the prominence of metal concentration in the central part compared to two other sites which are relatively near the continental input.

Thus the conclusion is that the Fe, Mn-rich layer is formed due to post-depositional migration of these elements during the Pliestocene/Holocene transitional periods because of emasculated influence of terrestrial input into these sites. Oxidation of labile organic matter provides the mechanism for the redox-related mobilization in the distal Bengal Fan.

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Erratum

Satellite data reveals pre-earthquake thermal anomalies in Killari area, Maharashtra

S. K. Srivastav, Manoj Dangwal, A. Bhattacharya and P. R. Reddy

[*Curr. Sci.*, 1997, **72**, 880–884]

Figures 2 and 3 on pages 882 and 883 have been interchanged though not the captions. We regret the error.

The Infamous Boundary – Seven Decades of Controversy in Quantum Physics. David Wick. Birkhaüser Verlag, AG Klasterberg, P. O. Box 133, CH-4010. Basel, Switzerland. 1996. Price: DM 88, SFr 78. 244pp.

John Stewart Bell was one of the keenest critics of the orthodox Copenhagen interpretation of quantum mechanics. He was also an exceptionally clear thinker and contributed decisively to the progress of the decades old debate on the meaning of quantum mechanics, and its bearing on the general notions of realism and locality in physics. Despite its stunning and practical successes – in applications to elementary particle physics, nuclear and atomic and molecular physics, condensed matter physics and chemistry, weak and electromagnetic and strong interaction phenomena – a basic problem for quantum mechanics has always been the location of the divide between the quantum microworld and the classical macroworld, the smooth transition from the one to the other, the relation between observed system and observing apparatus. In a famous 1971 article titled 'Introduction to the hidden-variable question', Bell referred to this hard-to-pin-down point of separation as 'the infamous boundary between classical and quantum worlds'.

David Wick's book is inspired not only in its title but also in its overall motivation and content by the spirit and the points of view expressed by Bell so sharply and strikingly on innumerable occasions. Wick's aim is to trace the early development of quantum mechanics; the struggles of the founding fathers in fashioning an interpretation; the emergence of the orthodox version based on Heisenberg's uncertainty principles and Bohr's complementarity principle; and then the attempts to construct alternative interpretations with an account of the generally hostile way in which these were received and treated by the 'establishment'.

An attractive feature of Wick's treatment is that the individual sections – twenty in all – are brief, focussed, each one devoted to one major theme or concept or historical development. Formal mathematics is kept at a minimum, technical points being relegated to numerous footnotes at the end of sections. In the beginning he sets the stage by recalling the evidence for the reality of atoms, the emergence of quanta, the magical discoveries of matrix and wave mechanics, and the ways in which Heisenberg and Bohr arrived respectively at the uncertainty and complementarity principles. Brief life sketches of Heisenberg, Schrodinger, von Neumann and Bell and the other principal characters are included at the appropriate places. Then follow accounts of events at the two Solvay Congresses of 1927 and 1930, which saw the opening of the Bohr–Einstein debate. At these two meetings, Einstein tried respectively to show that the position–momentum and the time–energy uncertainty principles could be 'beaten' – had it worked out, quantum mechanics or at least the proffered interpretation would have been shown to be inconsistent. The answers Bohr presented on the two occasions are well known. Einstein's third and final attack came in 1935 with the Einstein–Podolsky–Rosen argument – that quantum mechanics while possibly internally consistent gives an incomplete description of physical reality. On the way Wick recounts the von Neumann theorem of 1932: based on stated assumptions, no hidden variable extension of quantum mechanics restoring determinism is possible.

After this essentially historical recollection of the early period, Wick moves on to post-World War II developments. As he points out, while all along there had been some voices raised against the Copenhagen interpretation from the very beginning – witness Schrodinger and the early work of de Broglie apart from Einstein – the work of Bohm in 1952 and then by Bell beginning in the 1960s raised the intensity of the opposition, until today it seems child's play to express one's astonishment at and disagreements with the views of the founding fathers! The reviewer cannot help recalling a statement by Einstein in 1951 in a somewhat different context: 'All the fifty years of conscious brooding have brought me no closer to the answer to the question, "What are light quanta?" Of course today every rascal thinks he knows the answer, but he is deluding himself '.

Wick's treatment of Bell's theorems, the attempts to test them and the analysis of experimental results are illuminating and well-done. In particular the account of the ways in which Clauser, Shimony, Horne, Holt and Freedman each came to know of Bell's work, their individual motivations, and how they were drawn into collaborative efforts to test quantum mechanics is valuable and interesting. Loopholes in the interpretation of experiments displaying violations of Bell's inequalities are discussed. The concluding sections discuss the problems in finding a well-defined set of principles on which one can base quantum mechanics in its entirety, the philosophical standpoints of many of the leading figures, recent developments, and guesses for the future.

The book concludes with an excellently written elementary account of the relation of classical probability theory to quantum mechanics, and a derivation of the two Bell theorems, by William Faris.

Overall the book is stimulating and timely. It is an honest, irreverent and deliberately provocative account. (In contrast, the appendix is sober and to the point, avoiding all polemics). It does bring out the difficulty of the issues involved, and shows how easily one may succumb to prejudices of one kind or the other. However at several points the author seems needlessly harsh in his criticism of the ways in which the early pioneers expressed themselves and treated opposing views. He repeatedly comes down especially hard on both Bohr and Feynman, of course for different reasons. Beyond a point these diatribes, also against Heisenberg and Delbruck, do seem overdone. However one must grant the author's point that the Bohm interpretation of quantum mechanics, available since 1952, has not been given the attention it deserved, and for so long has been just ignored by so many. Wick argues that the 'establishment' adopted a generally bullying attitude to the opposition, at least partly because of its own underlying uneasiness on the foundations!

Speaking of the difficulty in overcoming prejudices, the reviewer could not help noticing that in one of Wick's parables, the two spies in the story are (of course) Boris and Natasha; it was the Buddha who abolished the real world; and Schrödinger's views could be respected and trusted just so far as he did not bring in Vedanta! In many places there is an avoidable flippancy and frivolity of expression, and sometimes factual errors. Thus while in 1925 Heisenberg took the 'leap beyond logic' and represented position and momentum by square arrays, one should remember that Born in 1924 had foreseen the role of 'two index quantities' in a future quantum mechanics. And Heisenberg's rule for multiplying arrays was motivated by the Ritz Combination Law of spectroscopy. Similarly the author of the time–energy uncertainty relation – so most of us have been taught – was Bohr and not Heisenberg. The discussion on the various alternative ways to understand the uncertainty principle is well motivated but finally leaves one uncertain. And Wick's confident assertion about Wigner – 'unlike most physicists, Wigner did not think the Copenhagen interpretation had settled the matter' – seems somewhat at variance with Wigner's own statement: 'The orthodox view is very specific in its epistemological implications. . . . A large group of physicists finds it difficult to accept these conclusions and, *even though this does not apply to the present writer*, he admits that the far-reaching nature of the epistemological conclusions makes one uneasy'.

In conclusion, the book is well worth reading to learn interesting points of history, to see the pressure of philosophical prejudices in every position or point of view, and to appreciate the utter nontriviality of the problems involved. One also understands that just as Bohr and Heisenberg were pioneers, so were Bohm and Bell in a later day. And in each case the courage and persistence shown by the pioneers made the way for those who followed deceptively easy.

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Yakov Ilich Frenkel: His Work, Life and Letters. Alexander S. Gilbergleit, ed. Birkhaüser Verlag, Postfach 133, CH-4010, Basel. 1996. Price: DM 198, SFr 168. 323pp.

Victor Frenkel's *Yakov Ilich Frenkel: His Work, Life and Letters* is a thoughtfully compiled biography of the great Soviet theoretical physicist, J. I. Frenkel (1894–1952) who lived and worked through what has come to be known as the golden age of the twentieth century physics. This biography is, however, not just about the many scientific achievements of J. I. Frenkel, the physicist, but also about Frenkel, the man (or J. I., as he is affectionately referred to throughout the book) – about his worldview and the civic convictions upheld by him through the tumultuous decades that followed the October revolution when the scientific ideas of Quantum Mechanics and the Relativity Theory were held suspect by the State.

In this book we are re-told of Frenkel's well-known contributions to theoretical physics which are deep and, indeed, far too many for any one individual. This fact stands acknowledged in terms of the diverse physical phenomena and effects that now bear his name. Thus, we have the Frenkel defects in crystalline lattices; the Frenkel excitons in semiconductors; the Frenkel–Kontorova equation for the plastic deformation giving Frenkel solitons; the Frenkel Saw, or the Double-Saw model for the elastic limit of crystals and for dry friction; the Frenkel-fields of quantum electrodynamics; and, of course, the Frenkel theory of the liquid state, the last mentioned being closest to his heart, and considered by many his abiding contribution to theoretical physics. Some others, however, viewed his idea of the exciton (the photo-excited mobile electron-hole pairs *sans* photoconductivity) as 'the pearl of his creative legacy'. Then there are the less known works traceable to Frenkel for which he did not quite get the credit that was rightly his. This includes his work on the superdense stars – the White Dwarfs – which, he had argued, were stabilized against gravitational collapse by the electronic degeneracy pressure, rather than by a high temperature as the great Eddington would have it. And similarly for his work on the theory of metals with 'collectivized' electrons obeying Pauli's 'residence law'; his theory of the superdense nuclear matter; or his dynamo theory of Earth's magnetic field. Even the electron-paramagnetic resonance (EPR) was in fact one of his sound theoretical guesses. So was his idea of the lattice-polaron. He had, perhaps, expressed these seminal ideas just in passing. Some of his ideas were simply ahead of his time, e.g., the Ioffe–Frenkel idea of tunneling was realized in Tunnel Diodes, long after his death.

The biographer has provided insight into the working of Frenkel's mind, his method, and his style of pursuing scientific enquiry. Frenkel belonged to 'the theoreticians of the experimentalist type'. His method was, we are told, 'to come forward with a kind of "catch" and then with its help to approach the truth'. He was known to have bemoaned the 'unhealthy passion for formal mathematical apparatus, a formal approach to physics that brings more damage than profit by tempting physicists to be satisfied with cheap mathematical trophies and to forget about the true essence of the problem considered'. The biographer hastens to clarify, however, that Frenkel was merely objecting to 'shooting guns at sparrows'. To him, introduction of new ideas remained the single greatest service one can render to science. It has been said of him that 'sometimes not even a model, but a precise, clear imaginative (if anthropomorphic, or terramorphic) expression by J. I. Frenkel, actually provided the formulation of some problem, later solved by other theoreticians'. This is well illustrated by his remark that '. . . the surface layer of every solid may be considered even in the absence of any alien adsorbed particles, as itself having been adsorbed with respect to the substrating mass'. One has only to read through his arguments as to why a liquid is kinetically closer to a solid than to the gas, that it naively resembles most, to be left in no doubt about its truth. The biography is, however, quite candid about some of Frenkel's intuitive remarks that may now seem untenable. Thus, for instance, Frenkel felt the gravitational field to be too 'macroscopic' to be quantized. But, precisely this and such otherwise inaccessible asides have added great value to this book for a discerning reader.

Last but not the least, this is a biography of an illustrious father by an affectionate son, not just filed from some archives but compiled with care from personal recollections and letters and familial anecdotes that go far beyond the usual public domain. Through reading these, a curious reader may even deconstruct the mind and the personality of J. I. Frenkel. From this record, J. I. emerges as a creative first-principle scientist, an encyclopaedic thinker, a kindly warm hearted professor with friends all over the world, and an artist. Some of the paintings/portraits by him reproduced in the book are simply disarming in their appeal. His students loved him, and there was no 'theoretical minimum' at his Physical-Technical Institute, that Landau, for one, would have insisted upon. For all his varied interests he was no great reader, or collector of books. But, he had read Jim Corbett's *Man-eaters of Kumaon* and *Leopard Man-eater from Rudraprayag*.

He was a prolific writer – with more than 200 papers published during 1916–1951 (some posthumously) and some 25 books, multi-printed since then. He considered his writing of books a commitment to science, even though, on his own admission, it did affect his research output adversely.

Finally, this book clearly belongs in any science library attached to a university/research institution. It belongs equally well in the personal collection of those with a sense for the history of science and the scientific ideas. Condensed matter physicists should love it. Personally, however, I would have liked the book to be somewhat shortened by selective deletion of some of the rather discursive letters.

N. KUMAR

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Nonlinearity and Chaos in the Physical Sciences

Editors: **Prof. R. Ramaswamy**, Jawaharlal Nehru University, New Delhi, and
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DEPARTMENT OF SCIENCE AND TECHNOLOGY

JSPS RONPAKU (DISSERTATION PH D) PROGRAMME 1998–99

Department of Science and Technology (DST) invites applications from promising and qualified young researchers for JSPS RONPAKU (Dissertation Ph D) Fellowships to Japan for 1998–99 offered by the Japan Society for the Promotion of Science (JSPS) within the framework of India–Japan Cooperative Science Programme (IJCSP). Four fellowships are available.

DESCRIPTION OF THE RONPAKU PROGRAMME

RONPAKU programme provides tutorial and financial support to researchers who wish to obtain their Ph D degrees from Japanese universities through the submission of a dissertation, without going through the course work.

Grantees under the RONPAKU programme are required to conduct their dissertation research under the joint supervision of a Japanese Advisor and an Indian Advisor. For this purpose, during the maximum period of five years, the RONPAKU Fellows will visit Japan once a year to conduct their research at a Japanese university/institute under the supervision of the Japanese Advisor who will visit the RONPAKU Fellow's country, when necessary, to supervise the research together with the Fellow's home Advisor.

FIELDS

All fields of Science & Technology.

ELIGIBILITY

Applicant should

1. be a citizen of India;
2. be holding a regular position as a researcher in a recognized scientific institution in India;
3. have already achieved substantial research results and, through dissertation research under the programme, be deemed likely to obtain Ph D degree successfully;
4. be able to forward the final application through proper channel of the parent institution.

JAPANESE ADVISOR

The Japanese Advisor must be either a professor, associate professor or lecturer who teaches in a doctoral programme of either a national, public or private university in Japan.

SUPERVISION OF THE RESEARCH

During the overall tenure of 5 years, the RONPAKU Fellow will visit Japan once a year for a maximum period of 90 days per visit to conduct research under the supervision of the Japanese Advisor. An extension of the Fellow's stay in Japan of up to 30 days may be approved in the final year of his/her fellowship tenure to obtain his/her Ph D degree.

The Japanese Advisor may visit the Fellow's country, not more than once a year (for a period of 7–30 days per visit) to supervise the research in accordance with the plans approved.

TERMS OF AWARDS

JSPS provides the following financial support:

To the RONPAKU Fellow

1. Round-trip economy class air ticket between India and Japan;

2. Maintenance allowance as per the JSPS regulations;
3. In Japan research trip allowance of up to Y 100,000;
4. Sickness and accident insurance coverage during the stay in Japan as per JSPS regulations;
5. Expenses related to dissertation submission and publication in the final year of the fellowship tenure, as per JSPS regulations.

To the Japanese host

1. Round-trip economy class air ticket between India and Japan;
2. Maintenance allowance for stay in India;
3. Supervising expenses (when receiving a RONPAKU Fellow);
4. Research allowances for supervising the RONPAKU Fellow;
5. Supervising expenses (when visiting Fellow's country)

APPLICATION PROCEDURE

The application forms can be obtained from the Programme Office, India–Japan Cooperative Science Programme (IJCSP), International Division, Department of Science & Technology, Technology Bhavan, New Mehrauli Road, New Delhi 110 016. Request for application should be received **latest by 15 August 1997 along with a self-addressed envelope (12 cm × 27 cm) entitled "JSPS RONPAKU Fellowship"**. The duly filled in application form should be forwarded by the candidate through proper channel, as applicable to his/her parent institution.

Application complete in all respects should reach the above address **latest by 15 September 1997**.

PHYSICS AND CHEMISTRY OLYMPIAD

(for Std XII students and below)

The Indian team may be participating at the 29th International Physics Olympiad to be held in July 1998 at Reykjavik, Iceland. The team members will be selected through 3 stages, namely, RPhO (Regional Physics Olympiad), INPhO (Indian National Physics Olympiad) and Summer Camp exams. RPhO is being tentatively planned for 2nd Sunday of December 1997. Students are advised to contact the address below for study materials and other information, while school principals are requested to write for the procedures, to make their school as an exam centre.

IChO (International Chemistry Olympiad) for 1998 will be held at Australia. Indians interested in chemistry may write for participation advice and more information.

Subhashish Chattopadhyay
20, N, Road, Bistupur
Jamshedpur 831 001
Phone: 0657–430166 (after 2200 hours)
Fax: 0657–427819 (always)
E-mail: auto@giascl01.vsnl.net.in
or
prodip@cse.iitkgp.ernet.in.

Government of India
Department of Atomic Energy

BOARD OF RESEARCH IN NUCLEAR SCIENCES

DAE YOUNG SCIENTIST RESEARCH AWARD

Applications are invited for the above award from exceptionally brilliant scientists below the age of 35 years. This award has been instituted to grant financial assistance to young scientists in their initial years of settling down in a career of research and development in Universities and Institutions of higher learning.

The Award carries a maximum research grant of Rs 5.00 lakh in a block of three years. This amount can be utilized for expenditure on equipment, consumable, travel and other contingent expenditure in connection with his/her programme.

Scientists/Engineers working and holding a regular position in universities/academic institutions of higher learning/research organizations are ONLY eligible to apply for this Award. The Award is given to those who have not received till the date of application any research grant from any agency.

Those working in the laboratories and institutions fully supported by government departments like DAE, DST, ICMR, CSIR and ICAR and in R&D organizations of Private Industry or fully supported by Private Industry are not eligible for the Award. Applicants who had earlier applied for the Award against our advertisement will not be considered for the Award.

Research projects/programmes in the following areas of interest to DAE will be considered for the award.

1. Physics
2. Chemistry
3. Life and Medical Sciences
4. Engineering Sciences
5. Material Science
6. Electronics and Instrumentation
7. Food and Agricultural Sciences
8. Radiation and Environmental Sciences
9. Radioisotope Applications.

There is no prescribed Application Form and **no last date for the receipt of application.**

Applicants are requested to send complete details along with their curriculum vitae, proposed research programme, names of possible referees who are conversant with the applicant's work and all other necessary information for proper evaluation of the applicant. The application should contain the following declaration: 'I hereby certify that I have never received any research grant from any funding agency as on date of application for this award.'

The application must be duly forwarded by the Head of the Institution along with the following certificate: 'This is to certify that Dr/Prof./ -------------------- is holding a regular position in our University/Institution. This University/Institution agrees to undertake the financial and other management responsibilities for the part of the research work which will be conducted in our University/Institution. We are also aware that 'No Overhead' charges will be paid to the University/Institution for carrying out this programme'.

15 copies of the completed application should be forwarded to:

R. S. BABU
Scientific Secretary
Board of Research in Nuclear Sciences
4th Floor, Central Complex,
Bhabha Atomic Research Centre, Mumbai 400 085
Tel: 022–551 8026, 022–556 3060 Extn. 2807, Fax: 91–22–556 0750

POORNA PRAJNA INSTITUTE OF SCIENTIFIC RESEARCH

ADMAR MUTT EDUCATION FOUNDATION, NO. 4, SADASHIVNAGAR
BANGALORE 560 080

POORNA PRAJNA POST-DOCTORAL RESEARCH POSITIONS

POORNA PRAJNA INSTITUTE OF SCIENTIFIC RESEARCH has been established by the Admar Mutt Education Foundation. This Foundation is a trust sponsored by the Admar Mutt Education Council, a registered Society, wholly devoted to the cause of Education. The Society is presently running 20 Poorna Prajna Education Institutions which have earned a name for themselves for providing quality education at school and college levels. The **Poorna Prajna Institute of Scientific Research** will be home for pursuit of internationally competitive research in basic sciences, an area of India's recognized strength. The Advisory Committee for the Institute comprises eminent senior scientists of our country.

In the first instance, applications as well as nominations are invited for the award of **four** prestigious **Post-Doctoral Research positions**, **two** of which will be **Post-Doctoral Fellows** and **two** will be **Research Scientists**. Fresh doctorates can apply for Post-Doctoral Fellowships with the initial consolidated monthly stipend of Rs 8000/-. Candidates with at least 2 years' post-doctoral experience will be eligible to apply for the positions of Research Scientist, which will initially carry a consolidated monthly stipend of Rs 12,000/-. The tenure of all **four** positions will be one year, extendable for two more years after review of research performance at the end of each year. The stipend for all post-doctoral fellows as well as research scientists will be raised by Rs 500/- per month after every year of acceptable performance.

Selected candidates will be provided with modest hostel-type accommodation and a contingency grant to support their research. In case accommodation is not provided, an allowance of Rs 1000/- per month will be given.

Candidates may indicate their speciality in any of the following disciplines.

1) Mathematics
2) Theoretical Studies in Physics, Chemistry or Biology
3) Materials Science
4) Information Science

Arrangements will be made by the Poorna Prajna Institute for selected candidates to be associated with eminent Professors at the Indian Institute of Science or other leading institutions in Bangalore until the necessary infrastructure becomes available at the Poorna Prajna Institute.

APPLICATIONS/NOMINATIONS are to be submitted to the **Hon. Director, Poorna Prajna Institute of Scientific Research, Admar Mutt Education Foundation, No. 4, Sadashivnagar, Bangalore 560 080 latest by 18 August 1997.** There is **no** prescribed form. Applications/nominations are to be accompanied by bio-data containing all essential information such as: Name & Address; Date of Birth; Marital Status; Educational Qualifications (mention University and Grade or % marks obtained) from the SSLC level to Doctorate (specialization in any of the disciplines mentioned above); Academic Distinctions; Research Publications/Reports; Synopsis of the Ph D thesis; Names of two Referees acquainted closely with the research of the applicant/nominee and any other relevant information. **Merit will be the sole criterion for selection and those with the brightest track record only may apply/be nominated.**

Current Science

SUBMISSION IN ELECTRONIC FORM

Authors who have been informed of acceptance of their manuscripts may send the final version in electronic form on floppy diskette (3.5" preferred; IBM PC format only, *not* Macintosh). The text of the manuscript only should be supplied as a plain ASCII file with no formatting other than line and paragraph breaks. (Wordstar 5.5 or 7.0 and Microsoft Word for Windows 6.0 are acceptable, but ASCII is preferred.) A hard copy of the text, with all typesetting information (italics, bold, mathematical type, superscripts, subscripts, etc.) must accompany the electronic copy. Tables and figures must be supplied only as hard copy. The diskette must be labelled clearly with the following: manuscript number, file name, file information (ASCII or Wordstar, version number, etc.).

Text may also be transmitted as ASCII only by e-mail to currsci@ias.ernet.in.

We expect that electronic submission will result in quicker processing for publication.

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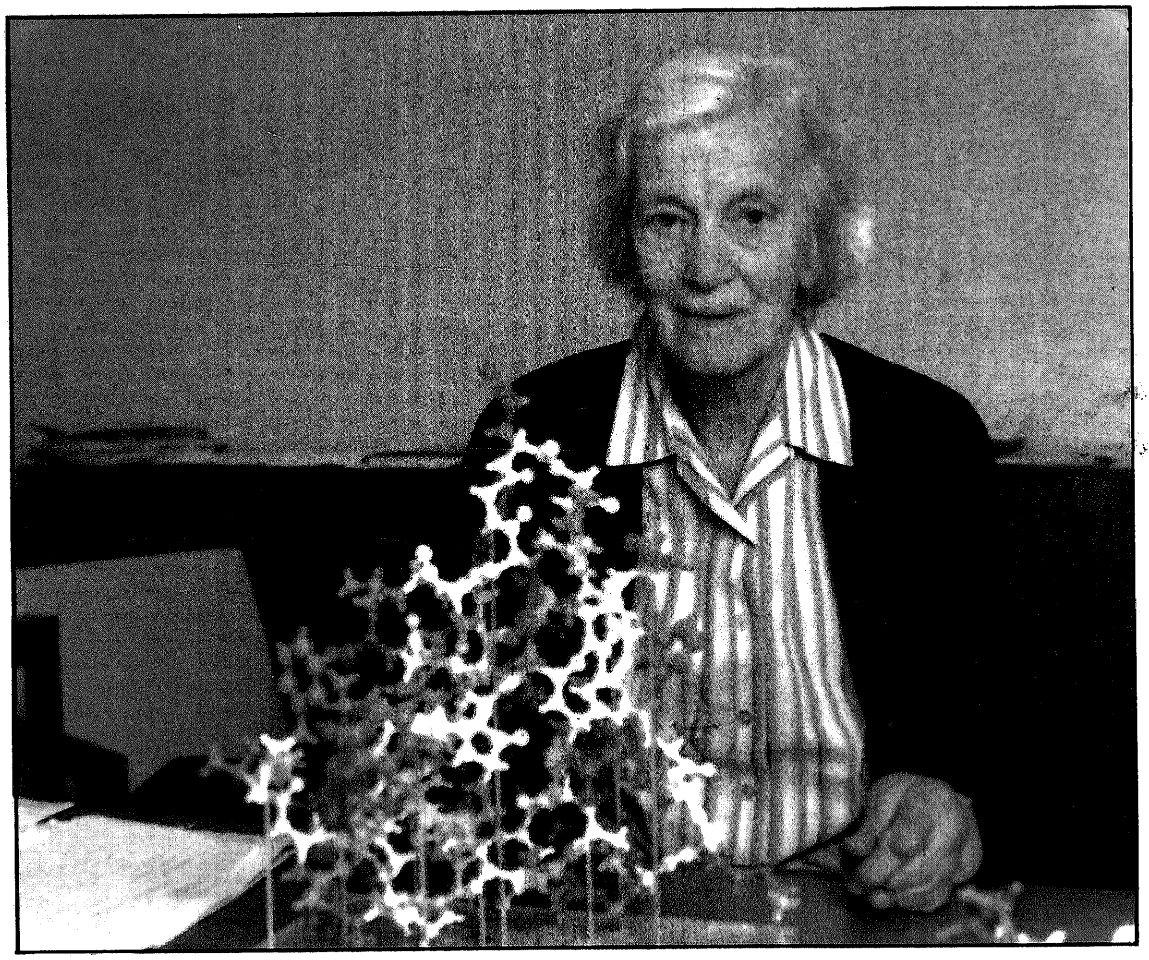

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